APPROVAL SHEET

Title of Thesis: THE ROLE OF CARDIOVASCULAR MUSCLE CELL Na^+-K^+ PUMP ACTIVITY IN THE DEVELOPMENT AND MAINTENANCE OF REDUCED RENAL MASS HYPERTENSION IN RATS

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ABSTRACT

Title of Dissertation: The Role of Cardiovascular Muscle Cell Sodium-Potassium Pump Activity in the Development and Maintenance of Reduced Renal Mass Hypertension in Rats

Stephen Joseph Huot, Doctor of Philosophy, 1981

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The mechanism of the elevated systemic arterial pressure in low renin experimental hypertension remains obscure. Recent data suggest that activity of the Na^+-K^+ pump is decreased in cardiovascular muscle cells of dogs with one-kidney, one wrapped and rats with one-kidney, one clip hypertension (low renin types of hypertension) due to a circulating ouabain-like agent. Because acute suppression of cardiovascular muscle cell Na^+-K^+ pump activity, by ouabain for example, increases blood vessel responsiveness and causes vasoconstriction, it has been hypothesized that suppressed pump activity may be responsible for development of hypertension in these animals. Additional data indicate that acute extracellular fluid volume expansion in dogs and rats stimulates release of a humoral ouabain-like factor which suppresses vascular Na^+-K^+ pump activity. Since the volume status of the hypertensive animals showing decreased pump activity is uncertain, the role of volume in release of ouabain-like factor in these animals is not clear. Therefore, in the present investigation, a low renin model of hypertension with documented volume expansion (reduced renal mass model) was studied. Using control normotensive and experimental hypertensive reduced renal mass rats, the objectives of this study were to: 1) assay blood from these animals for presence of a

humoral ouabain-like agent; 2) measure cardiovascular muscle cell Na^+-K^+ pump activity; 3) determine if there is a temporal correlation between suppression of vascular Na^+-K^+ pump activity and development of hypertension; and 4) investigate the role of the sympathetic nervous system and the AV3V region of the brain in the appearance of ouabain-like factor in blood, vascular Na^+-K^+ pump activity, and development of hypertension. Subtotally nephrectomized male Wistar rats (70-80 percent renal mass removed) were divided into pairs consisting of a control (distilled water drinking) and an experimental (one percent saline drinking) rat. All rats consumed a low sodium (0.02 percent) chow. systolic blood pressures and body weights were monitored weekly. After four weeks of sustained hypertension in the experimental rats and a similar time period in the paired controls, tail arteries and hearts were removed under pentobarbital anesthesia for measurement of vascular Na^+-K^+ pump activity (ouabain-sensitive ^{86}Rb uptake) and microsomal Na^+,K^+-ATPase activity, respectively. Additionally, aortic blood was collected for measurement of ouabain-like activity and/or serum or supernate composition. In another group of rats vascular Na^+-K^+ pump activity was measured at weekly intervals, following subtotal nephrectomy. In other rats, the effect of electrolytic lesions of the AV3V area or chemical sympathectomy (intravenous, intraventricular, or topical 6-hydroxydopamine) on release of humoral ouabain-like factor, vascular Na^+-K^+ pump activity, and development of hypertension were studied. Our results indicate that saline drinking induces hypertension in subtotally nephrectomized rats whereas restriction of sodium intake prevents the hypertension. Compared to normotensive controls, cardiovascular muscle cell Na^+-K^+ pump activity in

experimental hypertensive rats is decreased due to the action of a humoral ouabain-like agent, and the depressed vascular pump activity is not attributable to altered sympathetic innervation. Furthermore, there is a temporal association between pump suppression and development of hypertension, indicating a causal relationship. Central adrenergic pathways and the AV3V region are apparently involved in synthesis and/or release of the humoral ouabain-like factor as both intraventricular 6-hydroxydopamine and AV3V lesioning prevented (1) appearance of this factor in blood, (2) inhibition of vascular Na^+-K^+ pump activity, and (3) development of hypertension. These findings, in conjunction with our findings in other low renin models of hypertension, support the hypothesis that inhibition of cardiovascular muscle cell Na^+-K^+ pump activity by a humoral ouabain-like factor may be a common defect which is responsible for the elevated blood pressure in these animals.

THE ROLE OF CARDIOVASCULAR MUSCLE CELL SODIUM-POTASSIUM PUMP ACTIVITY IN THE DEVELOPMENT AND MAINTENANCE OF REDUCED RENAL MASS HYPERTENSION IN RATS

by

Stephen Joseph Huot

Dissertation submitted to the faculty of the department of Physiology
Graduate Program of the Uniformed Services University of the
Health Sciences in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy 1981

DEDICATION

To my wife Diane and to my parents

for their love and support.

ACKNOWLEDGEMENTS

A very sincere and special thanks goes to my major advisor, Dr. Moti Pamnani, for his guidance, support, and assistance throughout the course of this research project and during the preparation of this dissertation.

The author would also like to thank Dr. Francis J. Haddy for his guidance and advice on this dissertation project and for his valued input in the preparation of this thesis.

Also, I would like to thank Dr. David Clough and the other members of my advisory committee; Dr. Earl Ferguson, Dr. Tony Lo, and Dr. Doris Corcran for their input and support.

A special word of thanks goes to Ron Green, Marty Jagusiak, Tim Link, and Karen Knoble for their technical assistance and moral support. I am also grateful to Bob Whitmore, Mrs. Martha Mcshane, and Jessica Edwards for their technical assistance.

Also, I would like to thank Dr. John Bullard and Mrs. Jody Johnston for their assistance with university and departmental administrative matters.

Lastly, a sincere thanks goes to Mrs. Pat Prather, Barbara Siedman, and Ms. Charlene Gore for their secretarial assistance.

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INTRODUCTION

It is estimated that 23 million Americans have high blood pressure and thus are at a higher risk of stroke, heart attack, heart failure, renal disease or renal failure. Of these, 85 to 90 percent have essential hypertension, where the underlying cause of the increased pressure is unknown. At present, the etiology of essential hypertension is not understood.

To better understand the pathophysiology of essential hypertension a variety of animal models have been developed. Some of these are the result of genetic inbreeding, while others are due to investigator-induced alterations (functional or organic) of kidney function. However, in many of these experimental models of hypertension the pathophysiology of both the development and maintenance of the elevated blood pressure is not clear. The increased blood pressure can not be explained on the basis of altered levels of known vasoactive agents (renin-angiotensin-aldosterone system, catecholamines, kinins, prostaglandins, or vasopressin). In recent years, however, considerable evidence has been generated to indicate that total body sodium and/or body fluid volumes may play an important role in some low renin models of experimental hypertension and possibly in the pathogenesis of a proportion of essential hypertensive humans.

In this survey, literature regarding the role of total body sodium and body fluid volumes in the physiological mechanism of hypertension is reviewed.

ROLE OF SODIUM AND VOLUME IN THE PATHOGENESIS OF HYPERTENSION

In 1944 Kempner showed that hypertensive patients who were fed

a rice and fruit diet that was very low in sodium responded with a decrease in blood pressure and in edema (as evidenced by a drop in body weight). Since these early studies, much research has been done to determine the role of sodium and/or body fluid volumes in the pathogenesis of hypertension.

Role of Sodium

Many investigators have studied the effect of an increased dietary sodium intake on the level of arterial blood pressure.

Lenel et al. (1948) showed that when white leghorn chickens were provided with 1.2 percent saline instead of tap water for drinking their arterial blood pressure rose from an initial average value of 132/117 mmHg to a maximum average value of 182/152 mmHg in four to six days. Blood pressure remained at this level until the saline was discontinued.

Sapirstein et al. (1950) monitored weekly systolic blood pressure (tail plethysmography) for six weeks in rats drinking saline (1.5 to 2.5 percent) and in control rats drinking tap water. After a latency period of one to four weeks, systolic blood pressure was significantly higher in the rats drinking saline (122-138 mmHg) compared to those drinking tap water (99-103 mmHg).

Koletsky (1958) studied the effect of a chronically increased sodium intake on blood pressure in rats. White male rats weighing approximately 180 g were randomly divided into a control group (tap water for drinking) and an experimental group (one percent saline for drinking). Systolic blood pressure was monitored weekly (tail plethysmography). Blood pressure increased to hypertensive levels (over 150 mmHg) in 72 percent of the rats drinking saline after one to seven months of treatment.

Meneely and Dahl (1961) studied the relationship between dietary sodium intake and the incidence of hypertension in rats and in humans. In the rat studies, systolic blood pressures of animals consuming low (0.15-2.00 percent), moderate (2.80-5.60 percent), and excess (7.0-9.8 percent) sodium containing diets were monitored for nine months. Rats fed the low sodium diet remained normotensive (systolic blood pressure 122 mmHg) throughout the study while systolic blood pressures of the moderate and excess sodium fed groups increased to 130 mmHg and 152 mmHg, respectively by the ninth month. Also, the average life-spans of rats consuming the moderate and excess sodium diets were significantly shorter than rats fed the low sodium chow. In their human studies, Meneely and Dahl reported a correlation between sodium intake and the incidence of hypertension in humans. Five groups of peoples with different average daily sodium intakes were studied: Eskimos (4 g sodium/ day); Pacific Marshal Islanders (7 g sodium/ day); North Americans (10 g sodium/day); Southern Japanese (14 g sodium/day); and Northern Japanese (26 g sodium/day). The incidence of hypertension (diastolic blood pressure > 90 mmHg) in each of these groups were 0.0, 6.9, 8.6, 21.0, and 39.0 percent, respectively. These investigators concluded that in both rats and humans there is a correlation between the level of dietary sodium intake and the incidence of hypertension.

Vogel (1965) progressively increased the dietary sodium chloride intake of normal dogs to 2 g/kg body weight over a 12-day period, while providing 0.9 percent saline for drinking. The animals then maintained this level of sodium chloride intake for an additional 14 days. Mean arterial blood pressure was significantly elevated after 10 to 12 days of salt feeding and had risen from a control level of 109 ± 5 mmHg, to

a level of 130 ± 4 mmHg by the 26th day of salt feeding. The hypertension was sustained as long as the salt intake was maintained.

Freis (1975) has reviewed several epidemiological studies relating the incidence of hypertension in humans with the level of sodium intake. He notes that hypertension is not found in "unacculturated" societies (such as rural Uganda, the Amazon Basin, and the San Blas islands of Panama) and that the blood pressure does not rise with age as it does in "acculturated" societies. Also, the sodium ingestion in these "unacculturated" societies is much lower than in the "acculturated" societies and the incidence of hypertension correlates well with the level of sodium intake.

Together, these studies show that it is possible to raise the blood pressure of normal animals by increasing their sodium intake. In addition, there seems to be a correlation between the incidence of hypertension in humans and the level of dietary sodium intake.

Role of Body Fluid Volumes

Since an increase in total body sodium is accompanied by increased fluid retention, several investigators have studied the role of volume in the pathogenesis of hypertension. Because sodium ingested in the diet is distributed predominately in the extracellular space, particular emphasis has been placed on the relationship between extracellular fluid volume and arterial blood pressure.

Murphy (1950) measured changes in plasma volume, extracellular fluid volume, and arterial blood pressure in hypertensive patients treated with the Kempner rice diet (daily sodium intake of less than 8 mEq). Plasma volume decreased by 10 percent and extracellular fluid volume decreased by 12 percent 10 to 14 weeks after the patients began

the diet. In all cases, both systolic and diastolic blood pressure decreased although there was no simple quantitative relationship between these volume changes and the changes in arterial pressure.

Conway (1966) measured cardiac output and arterial blood pressure changes via chronic indwelling right atrial and carotid artery cannulas, respectively, in conscious dogs during acute volume expansion. Intravenous infusion of 100 ml of blood plus 50 ml of dextran (to minimize the expected change in hematocrit) caused cardiac output to rise, with no initial effect on blood pressure. However, two hours after the infusion, blood pressure was significantly elevated, cardiac output had returned to normal, and peripheral resistance was significantly elevated.

Norman et al. (1975) investigated the role of increased extracellular fluid volume on arterial blood pressure in bilaterally nephrectomized sheep that were maintained by chronic dialysis. At the time of nephrectomy, an arteriovenous shunt of silastic tubing was inserted into the right renal artery and right renal vein, and then extended about 15 cm into the aorta and vena cava, respectively. The shunt was exteriorized through the abdominal muscle and used for dialysis. Extracellular fluid volume was then expanded by 20 percent by the intravenous infusion of saline and this was then maintained for seven days by adjusting the dialysis. Initially there was only a slight increase in blood pressure. However, after four days of volume expansion, mean arterial blood pressure had risen from a pre-expansion value of 95 mmHg to 134 mmHg.

Data from these sodium and volume studies indicate that changes in extracellular fluid volume and/or exchangeable body sodium affect blood pressure. Since the time required for this effect to occur is

from several hours to several days, the increased blood pressure appears to be the result of some slow and indirect effect of the change in extracellular fluid volume and/or sodium.

Importance of Volume vs. Sodium

Whether sodium retention causes hypertension entirely as a result of a sodium-induced increase in volume or whether increased exchangeable body sodium per se can also elevate arterial pressure by an effect of sodium ions on vascular resistance has been studied by several investigators.

Haddy and Scott (1971) measured blood pressure changes in anesthetized dogs during acute selective sodium chloride depletion without water depletion. Dogs were anesthetized with sodium pentobarbital and injected intravenously with furosemide (20 mg/kg) and urine flow was monitored. In the experimental group, the amount of water lost in the urine was replaced with an equal amount of isosmotic modified Ringer's solution in which mannitol had been substituted for sodium chloride and sodium bicarbonate. A femoral artery cannula was used to monitor arterial blood pressure and to sample arterial blood for measurement of plasma sodium and osmolality. Plasma sodium concentration fell 29 mEq/L in 76 minutes, while plasma osmolality did not change. Control dogs were similarly prepared but mannitol was not subsituted for sodium and consequently neither plasma sodium nor osmolality changed during the experiment. Mean arterial blood pressures rose 14 and 12 mmHg in experimental and control groups, respectively. In this study, acute sodium chloride depletion per se did not affect blood pressure.

Finnerty et al. (1970) showed that when five percent glucose in water was intravenously infused into hypertensive patients, mean arteri-

al pressure rose 15 to 20 percent in 60 to 90 minutes. Since plasma sodium concentration was not increased, the observed changes in blood pressure during dextrose infusion were the result of changes in extracellular fluid volume and were not dependent on changes in extracellular sodium.

Norman et al. (1975) prepared unilaterally nephrectomized sheep for dialysis by removing the right kidney and inserting an arteriovenous shunt into the right renal artery and right renal vein that was then exteriorized through the abdominal wall. Each animal was then given daily intramuscular injections of deoxycorticosterone (25 mg) in oil for eight days. Plasma sodium concentration was then increased by 18 mEq/L over a four-hour period by dializing against a physiologic solution containing a high sodium concentration. The elevated plasma sodium level was then maintained for seven days by restricting the animal's fluid intake (such that extracellular fluid volume did not increase) while continuing the deoxycorticosterone injections. This significant elevation of plasma sodium concentration caused only a slight (4 mmHg) increase in mean arterial blood pressure.

Manning et al. (1979) investigated the cardiovascular effects of intravenously infused sub-pressor amounts of ADH (0.067 mU/Kg/min in 26 ml of isotonic saline) plus hypotonic saline (153.0 ml/Kg/day) over a 14 day-period in dogs in which 70 percent of the renal mass had been removed. The saline was diluted or concentrated within a range of 66.8 to 120.0 mEq/L. Extracellular fluid volume and mean arterial blood pressure were significantly increased by the second day of infusion while plasma sodium concentration was decreased.

Collectively, these data show that changes in plasma sodium con-

centration per se does not affect blood pressure whereas changes in extracellular fluid volume does.

HEMODYNAMICS OF VOLUME-DEPENDENT HYPERTENSION

The two immediate determinants of arterial blood pressure are cardiac output and total peripheral resistance, as given by the relationship:

Mean Arterial Pressure = Cardiac Output x Total Peripheral Resistance

An increase in cardiac output and/or total peripheral resistance will cause an elevation of blood pressure.

In the chronic stages of several models of experimental hypertension, as well as in the established stage of essential hypertension in humans, cardiac output is normal or low while total peripheral resistance is elevated. Ferrario (1974) measured cardiac output and total peripheral resistance during the development of one-kidney, one clip hypertension in dogs. Cardiac output rose within 48 hours after renal artery constriction and remained elevated for up to 28 days. Blood pressure rose with the increasing cardiac output while total peripheral resistance remained normal or fell slightly. Peripheral resistance then increased, cardiac output returned to normal, and the elevated blood pressure was maintained. Similar findings have been reported by Coleman and Guyton (1969) in dogs with reduced renal mass-salt hypertension. Additionally, Frohlich et al. (1971) have shown that cardiac output is normal or reduced in patients with established essential hypertension while the increased arterial pressure is maintained by increased peripheral resistance.

These studies indicate that the primary hemodynamic alteration responsible for maintenance of the elevated arterial pressure in estab-

lished hypertension is increased peripheral resistance. Total peripheral resistance is itself dependent on viscosity of the blood and geometry of the blood vessels as described by Poiseulles law:

$$R = \frac{8nl}{r^4}$$

Where R = resistance, n = viscosity, l = length, and r = radius.

The role of blood viscosity in hypertension has not been extensively studied. There is some evidence which indicates that blood rheology may be abnormal in certain forms of hypertension. Blood viscosity at a given temperature depends on cell concentration, cell deformability, cell aggregation, and plasma viscosity.

Chien (1977) studied blood rheology in patients with essential hypertension. The data were analyzed according to plasma renin activity. Blood viscosity in low renin, essential hypertensive patients was not significantly different from normotensive control patients, but patients with high renin activity showed significantly increased blood viscosity compared to the controls. The increased blood viscosity in these patients was attributed to a high plasma viscosity and reduced RBC deformability. In the low renin patients plasma viscosity was increased, but this was compensated for by a low hematocrit resulting in a normal blood viscosity.

This study indicates that changes in blood rheology may play a role in hypertension, but evidence is inconclusive. Other parameters which affect peripheral resistance have been investigated in more detail.

In hypertension, alterations in blood vessel geometry, particularly radius, appear to be of primary importance. Changes of vascular

radius can be affected by active or passive mechanisms. These active mechanisms include neurohumoral, local metabolic, and myogenic influences. The passive changes may be due to blood vessel waterlogging and hypertrophy.

Mechanisms of Increased Peripheral Resistance

The factors responsible for the changes in blood vessel radius and therefore peripheral resistance in hypertension are unknown. Nonetheless, several hypotheses have been advanced to explain the chronically elevated peripheral resistance seen in hypertension. These include: long term whole-body autoregulation; altered vascular morphology; and alterations in the concentration of circulating vasoactive agents.

Long Term Whole-Body Autoregulation. Coleman annd Guyton (1969) investigated the effect of chronic volume expansion on cardiac output and total peripheral resistance in reduced renal mass (70 percent renal mass removed) dogs. Two groups of experiments were performed. In the first group, isotonic saline was substituted for drinking water and in the second group isotonic saline (3.72 L/day) was continuously infused through an indwelling right atrial catheter. In both groups the initial hemodynamic alteration was an increase in cardiac output, reaching a maximum (140 and 129 percent of control in the first and second groups, respectively) three to four days after treatment began. Cardiac output then progressively fell and returned to control levels by the tenth day of saline treatment. Total peripheral resistance decreased over the first two to three days and then rose to a level 167 percent above baseline. Mean arterial blood pressure rose continually from a control value of 105 mmHg to 140 mmHg by the seventh day and

remained at this level throughout the experiment. These investigators concluded that in partially nephrectomized dogs, saline loading results in elevated arterial blood pressure that is initially caused by increased cardiac output, but is eventually sustained by increased peripheral resistance.

Based on this data Guyton proposed the hypothesis of long term whole-body autoregulation. This hypothesis states that "Overperfusion, or increased cardiac output, if it is maintained for an adequate length of time, causes an autoregulatory response that increases total peripheral resistance and arterial pressure" (Coleman et al., 1971).

Ferrario (1974) investigated the correlation between arterial blood pressure, cardiac output, and total peripheral resistance in one-kidney, one clip hypertensive and control dogs. Mongrel dogs of either sex were unilaterally nephrectomized and an externally adjustable clamp placed around the opposite renal artery. Four weeks later a catheter was inserted into the iliac artery for continuous measurement of arterial blood pressure. Baseline values were recorded and the previously implanted clamp was constricted sufficiently to produce hypertension. Daily hemodynamic measurements were then taken for seven weeks. Mean arterial blood pressure and cardiac output rose within one to ten days following renal artery constriction. Peripheral resistance did not change during this time period, but became elevated 14 to 21 days after surgery. With time, cardiac output fell to control levels while total peripheral resistance and arterial blood pressure remained elevated. Ferrario concluded that an initial increase in cardiac output plays an important role in the development of hypertension due to renal artery constriction and that increased total peripheral resistance is

important in the maintenance of this hypertension.

These data are quite similar to those reported by Coleman and Guyton (1969) in saline-loaded reduced renal mass dogs and therefore support Guyton's hypothesis of long term whole-body autoregulation.

Hawthorne et al. (1974) measured changes in left ventricular myocardial contractility in unilaterally nephrectomized dogs at 24 hour intervals for 144 hours after constriction of the renal artery of the sole remaining kidney. The average dp/dt was significantly increased above control values from the 48th hour through the 120th hour after left renal artery constriction and had nearly returned to control values by 144 hours. Mean arterial blood pressure was significantly elevated by 48 hours after renal artery constriction and remained elevated throughout the study. Total peripheral resistance increased in parallel with the blood pressure and it also remained elevated.

Nivatipumin et al. (1975) bilaterally nephrectomized male Wistar rats (250-300g) and 24 hours later studied cardiac contactility, as measured by systolic pressure and dp/dt, of the isovolumetrically contracting heart during acute aortic occlusion. Myocardial contractility was increased in these acutely uremic rats even when phenoxybenzamine was given to block the rise in arterial blood pressure that accompanies bilateral nephrectomy. In a similar study, Scheuer et al. (1975) found that cardiac contractility was increased 48 hours after 5/6 nephrectomy of male Wistar rats that were fed a normal sodium diet.

These studies indicate that increased cardiac contractility may play a role in the increased cardiac output observed in the early stages of several models of volume-dependent hypertension. These studies could help explain how increased volume causes increased cardiac output.

All of these data indicate that both cardiac output and total peripheral resistance participate in the genesis and/or maintenance of volume-dependent models of hypertension. The observed sequence of hemodynamic changes in hypertension, an initially increased cardiac output which subsequently returns to control levels as peripheral resistance rises and blood pressure increases, supports Guyton's hypothesis of overperfusion leading to long term whole-body autoregulation.

There are however several reasons for questioning whether long term whole-body autoregulation plays a role in the mechanism of the elevated peripheral resistance in hypertension.

Haddy and Overbeck (1976) noted that the classic autoregulatory phenomenon has a time course that is measured in seconds or minutes, whereas the elevated peripheral resistance seen in volume expanded hypertension has a time course that is measured in days.

However, Coleman et al. (1971) argue that the nervous system (via the baroreceptors) delays onset of the whole body autoregulatory response by reflexly lowering peripheral resistance in response to the initially increased cardiac output and increasing arterial blood pressure. With time this reflex is overcome by the autoregulatory response and peripheral resistance increases as the blood pressure rises further and both remain elevated.

This concept of autoregulation delayed in onset by the nervous system is refuted by the studies of Conway (1966). He observed that the time course of increased resistance following the infusion of 100 ml of blood plus 50 ml of dextran into the conscious dog was not different after autonomic blockade with pentolinium (7.5 mg iv plus 7.5 mg subcutaneous). Initially, the increased volume caused an increase in

cardiac output without affecting peripheral resistance as mean aortic blood pressure rose slowly. One and one-half hours later cardiac output returned to normal and total peripheral resistance was elevated. Furthermore, Andresen et al. (1981) have shown that resetting of the arterial baroreceptors occurs after sustained alterations of blood pressure of only 15 minutes duration. They concluded that rapid peripheral resetting of the arterial baroreceptors limits the duration and magnitude of the reflex autonomic effects elicited by sustained alterations in blood pressure.

These studies indicate that the increased peripheral resistance seen in volume-dependent models of hypertension cannot be attributed to classic autoregulation delayed in time by the nervous system.

Several other studies also indicate that mechanisms other than long term whole-body autoregulation may be responsible for the increased peripheral resistance in these models of hypertension.

Vyden et al. (1972) studied resistance in the peripheral vasculature of five anephric and ten normal patients. Total peripheral resistance was increased in the anephric subjects compared to the normals and the increased resistance was greater in cutaneous vessels than in the muscle vascular bed. Since the skin vascular bed is known to exhibit weak autoregulation, this observation does not support the concept of long term whole-body autoregulation.

Onoyama et al. (1974) studied the hemodynamic events of mineralcorticoid hypertension in dogs. Trained conscious dogs with chronically implanted iliac vein catheters were maintained on sodium restricted diets (1.4 mEq Na^+/day) and divided into two groups. One group received metyrapone orally (100 mg/Kg/day) while the other group received

nothing. Each of these groups were then divided in half; one half received 10 mEq/day of supplemental sodium and the other half received 60 mEq/day. The supplemental sodium was administered by slow intravenous infusion of 0.9 percent sodium chloride solution for 7 or 14 days. This created groups of dogs (metyrapone treated and untreated) with cummulative sodium intakes of 70, 140, 420 and 840 mEq. Cardiac output, blood pressure, extracellular fluid volume, and plasma volume were measured daily and total peripheral resistance was calculated daily. None of these parameters were significantly different in the metyrapone treated and untreated groups prior to supplemental sodium administration. With increasing cummulative sodium intake plasma and extracellular fluid volumes increased in both the treated and untreated groups, although the increases were limited at the higher levels of sodium intake due to natriuresis. Mean arterial blood pressure rose significantly in treated dogs with cummulative sodium intakes of 420 and 840 mEq, while it remained unchanged in all untreated dogs. Cardiac output rose to similar levels in all dogs with supplemental sodium. Total peripheral resistance, however decreased in the untreated group at the two highest levels of sodium intake, whereas in the treated group total peripheral resistance rose in parallel with arterial blood pressure at the two highest levels of sodium intake. Since changes in cardiac output and fluid volumes were virtually identical in all groups at each level of sodium intake, these findings indicate that changes in blood flow were not responsible for the subsequent rise in arterial blood pressure.

These investigators concluded that this steroid, salt, and water dependent hypertension is resistance mediated from its early stages and

cannot be explained by Guyton's hypothesis of overperfusion long term whole-body autoregulation.

Mark et al. (1975) studied the effect of low and high sodium intakes on arterial blood pressure, forearm vascular resistance, and blood flow in borderline hypertensive subjects. Subjects were studied after 10 days of low sodium intake (10 mEq/day) and after 10 days of high sodium intake (410 mEq/day). Mean arterial blood pressure of patients on low sodium intake was 89 ± 3 mmHg whereas after the high sodium intake period the average blood pressure was 98 ± 2 mmHg. Forearm vascular resistance changed from 13.5 ± 2.2 units while on the low sodium diet to 19.1 ± 3.0 units on the high sodium diet, while forearm blood flow fell from 7.8 ± 1.2 to 5.9 ± 0.8 ml/min x 100 ml when the patients switched from the low to the high sodium diets. The findings of increased resistance with decreased blood flow in the high sodium group and decreased resistance with increased blood flow in the low sodium group are not compatible with the concept of overperfusion autoregulation.

Conway and Hatton (1978) measured cardiac output, total peripheral resistance, and arterial blood pressure during the development of one-kidney, DOCA, salt hypertension in dogs. Two groups of dogs were studied. In group I dogs hemodynamic parameters were measured on days 3, 7, and 14 following subcutaneous implantation of deoxycorticosterone (75 mg/kg) and substitution of a one percent NaCl solution containing 0.25 percent KCl and 0.25 percent sugar for drinking. Group II dogs received the same treatment plus 10 mg/kg atenolol (a β adrenergic blocker) orally twice daily throughout the study and hemodynamic parameters were also measured on days 3, 7, and 14. Systolic and diastolic blood pressures increased in both group I and group II dogs within seven

days after saline drinking began and remained elevated throughout the study. The blood pressures of group I and group II dogs reached approximately the same level (182/111 mmHg and 189/113 mmHg, respectively). In group I dogs, both cardiac output and total peripheral resistance showed small increases, this combination being responsible for the development of hypertension. In group II dogs, cardiac output was significantly reduced during the first seven days of saline drinking and was further reduced at day 14. Total peripheral resistance increased progressively over the 14-day period and was responsible for the elevated blood pressure in this group. Conway and Hatton concluded that the development of one-kidney, DOCA, salt hypertension does not depend on an initial elevation of cardiac output and subsequent autoregulation.

The evidence presented above indicates that long term whole-body autoregulation cannot explain the increased peripheral resistance seen in some models of hypertension. These observations have led to the investigation of other mechanisms which may contribute to the elevated peripheral resistance in hypertension.

Changes in vascular morphology. It is well known that there are morphological changes of the arterial vasculature associated with hypertension. These changes include medial hypertrophy, fibrosis, and waterlogging. Several investigators have proposed that changes in vascular morphology are responsible for the elevated peripheral resistance seen in hypertension.

Using a constant flow isolated hindquarters preparation, Folkow et al. (1973) measured changes in vascular resistance during maximal vasodilation and during infusion of norepinephrine in spontaneously hypertensive rats (SHR) and normotensive control rats (NCR). Resistance

during maximal vasodilation was found to be greater in SHR than in NCR. Also, while the threshold dose of norepinephrine required to increase resistance was the same in SHR and NCR, the dose response curve was steeper and obtained a greater maximal resistance in SHR compared to NCR. These investigators concluded that the increased peripheral resistance and hemodynamic responses seen in SHR can be ascribed to an increased vessel wall/lumen ratio caused by medial hypertrophy. Such structural changes appear to be crucial for the genesis and maintenance of hypertension.

Hutchins and Darnell (1974) studied blood vessel length and number in the microvascular circulation of the creamaster muscle of SHR and control rats. There were no significant differences in blood vessel length between SHR and control rats. There was, however, a significant reduction in the total number of small arterioles in the hypertensive animals to approximately 50 percent of the average number of vessels observed in the normotensive animals. These investigators did not measure vessel wall/lumen ratios.

Neubig and Hoobler (1975) prepared a group of one-kidney, one clip hypertensive rats and five weeks later studied the effect of unclipping the renal artery on blood pressure. Unclipping was studied in two groups of rats. In both groups mean arterial blood pressure was monitored in the conscious state via an indwelling carotid artery cannula for six hours after removal of the renal artery clip. In group I, saline was infused to exactly replace urine volume loss. In group II there was no fluid replacement. Mean arterial blood pressure fell from 135 mmHg before unclipping to 107 and 100 mmHg two hours after unclipping for groups I and II, respectively, and remained at this level

throughout the six-hour period.

Muirhead et al. (1976) reported similar findings in one-kidney, one clip hypertensive rats. Unclipping of the renal artery lowered blood pressure to normal in three hours if there was no volume replacement, and in 20 hours if fluid loss was prevented by anastomosing the ureter and vena cava.

Although it is apparent that structural changes play a role in the maintenance of hypertension, it seems unlikely that vascular restructuring could occur rapidly enough to account for the genesis of the elevated resistance seen in some volume expanded models of hypertension (eg. Conway, 1966, observed increasing blood pressure and peripheral resistance one and one-half hours after the intravenous infusion of blood plus dextran). Also, the studies by Neubig and Hoobler (1975) and by Muirhead et al. (1976) which showed reversal of one-kidney, one clip hypertension three hours after unclipping of the renal artery, further indicate that factors other than changes in vascular morphology play an important role in the maintenance of hypertension.

Altered Concentration of a Circulating Vasoactive Agent. Another hypothesis which has been proposed to account for the elevated peripheral resistance in hypertension, is an altered concentration of a circulating vasoactive agent. A number of studies support this hypothesis.

Solandt et al. (1940) cross circulated blood between dogs with either one-kidney, one clip or one-kidney, one wrap hypertension and bilaterally nephrectomized, smaller, normotensive assay dogs to determine whether blood from the hypertensive animals contained a pressor agent. Blood pressure of the nephrectomized assay dogs rose during cross circu-

lation while blood pressure of the hypertensive dogs fell slightly or did not change. The pressor response of the assay animal began approximately one hour after cross circulation began. These investigators concluded that blood from dogs with these models of hypertension contains a substance which raises blood pressure of recipient, nephrectomized dogs.

Gordon et al. (1953) cross circulated blood between sodium pentobarbital anesthetized rabbits with one-kidney, one clip hypertension and normotensive rabbits which had been salt-loaded by drinking two percent saline for three or more weeks. Cross circulation between the carotid artery of one rabbit and the jugular vein of the other was maintained for 30 to 60 minutes. Blood pressure was then monitored for six hours. Systolic blood pressure of the salt-loaded rabbits rose an average of 25 mmHg two to three hours after completion of the cross transfusion. Blood pressure of the hypertensive animals did not significantly change.

Dahl et al. (1969) measured blood pressure in parabiotically joined salt-resistant (R) rats and salt sensitive (S) rats. S rats become hypertensive when fed a high sodium diet (8 percent) and remain normotensive when fed a low sodium diet (0.4 percent). R rats remain normotensive when fed either diet. Two series of experiments were carried out. In one series blood pressure changes were monitored in parabiotically united S and R rats consuming a high sodium diet. In the second series blood pressure was recorded in parabiotic S and R rats consuming low sodium diets. In this study the S rats had undergone unilateral nephrectomy followed by clipping of the opposite renal artery before being parabiotically joined to the R rats. In the first series of experiments, mean arterial blood pressure rose significantly in R

rats within two weeks of parabiosis and was 172 mmHg at the end of the study (six months). Also, the rate of development and average level of hypertension was lower in these parabiotic S rats than in non-parabiotic S rats. In the second series of experiments both animals consumed the low sodium diet. This resulted in the development of hypertension in both the S and R rats. These investigators concluded that some humoral factor which modifies blood pressure passed the parabiotic junction.

Michelakis et al. (1975) assayed plasma of patients with malignant hypertension as well as plasma of dogs with one-kidney, one clip hypertension for the presence of a sensitizing factor to pressor agents. Venous blood was collected from hypertensive and control normotensive dogs and from hypertensive and control normotensive patients and kept in cold siliconized tubes containing EDTA. Plasma was then separated by centrifugation and tested for sensitizing activity. Pentolinium-treated bilaterally nephrectomized rats were used as bioassay animals. Under sodium pentobarbital anesthesia, catheters were inserted into a carotid artery and a jugular vein for recording blood pressure and injecting samples, respectively. Responses to 0.4 ng of intravenously injected angiotensin II were then recorded. After blood pressure returned to the baseline (mean blood pressure 95 mmHg) and stabilized, 15-20 ul of a plasma sample was injected. Repeated intravenous injections of 0.4 ng of angiotensin II were then given at three to five-minute intervals and changes in sensitivity to angiotensin II noted. Sensitivity to angiotensin II was increased an average of 42 and 39 percent by plasma from hypertensive dogs and hypertensive patients, respectively. This study supports the hypothesis that a circulating sensitiz-

ing factor is present in plasma from hypertensive patients and animals and that this factor may be involved in the pathogenesis of hypertension.

Self et al. (1976) investigated the effect of intravenously injected hypertensive rat serum on vasopressor responsiveness to norepinephrine. Three groups of rats were prepared. Group I rats were fed a diet containing 1.3 percent sodium chloride while groups II and III were fed diets containing 5.6 and 8.4 percent sodium chloride, respectively. Systolic blood pressure was monitored weekly and after 225 days on the diet, blood was collected by decapitation and serum separated by centrifugation. The serum was then tested for sensitizing activity in bilaterally nephrectomized pentolinium-treated assay rats. The assay rats were anesthetized with dial urethane, a norepinephrine dose-response curve generated, and the quantity of norepinephrine required to produce a 10 mmHg rise in mean arterial blood pressure (referred to as the standard dose) determined. Fifteen to 25 ul of serum sample from one of the sodium fed rats was then injected into an assay rat through a jugular vein cannula and the standard dose of norepinephrine was repeatedly injected at six-minute intervals until no further increase in vasopressor response occured. Animals from the three diet groups were then classified as hypertensive (systolic blood pressure > 142 mmHg) or as normotensive. Eleven percent of group I rats became hypertensive and 47 percent of groups II and III rats became hypertensive. Injection of sera from hypertensive rats augmented responses to the standard dose of norepinephrine by an average of 29 percent, while sera from normotensive rats did not significantly alter the response to norepinephrine. These investigators concluded that a humoral

agent is present in the serum of hypertensive animals which augments their response to vasopressor agents and may play a role in the hypertensive process.

Collectively, these experiments strongly suggest the presence of a humoral factor or factors which may be involved in the pathophysiology of some volume-dependent models of hypertension. This factor is probably not one of the known vasoactive agents (i.e. norepinephrine, renin, antidiuretic hormone, prostaglandins, or kinins) as their concentrations are not altered in a direction which would contribute to the hypertension (Haddy and Overbeck, 1976).

There are technical problems which currently prevent accurate measurement of plasma kinin and prostaglandin levels in hypertensive and normotensive animals or in salt loaded normal animals which make comparisons difficult (Levinsky,1979; Frolich and Walker,1980). Furthermore, a lack of these vasodilators would not be congruent with bioassay studies which suggest the presence of a vasoconstrictor substance.

Plasma renin activity and angiotensin II concentrations have been shown to be normal or low in animals with volume expanded hypertension. Ylitalo and Gross (1979) and Pitcock et al. (1980) have reported low plasma renin activity in rats with subtotal nephrectomy (70-80 percent renal mass removed)-salt hypertension. Brunner et al. (1971) found that angiotensin II antibodies, as well as synthetic angiotensin II inhibitor (sarcosine^{1} Ala^{8}-angiotensin II), failed to significantly lower the blood pressure of rats with one-kidney, one clip hypertension. Bumpus et al. (1973) observed that intravenous infusions of angiotensin II inhibitors failed to lower blood pressure in rats with chronic one-kidney one clip hypertension or in dogs with one-kidney, one clip or

one-kidney, one wrapped hypertension. It seems unlikely that the renin-angiotensin system plays a significant role in the maintenance of elevated blood pressure in these volume-dependent models of hypertension.

Vasopressin also does not appear to play a significant role in the mechanism of volume-dependent hypertension. Crofton et al. (1979) observed that urinary excretion of vasopressin is increased in rats with one-kidney, DOCA, salt hypertension and that infusion of vasopressin antagonists significantly lowers blood pressure of the hypertensive rats. In contrast to this finding, Rabito et al. (1980) found that infusion of vasopressin antagonists does not lower the blood pressure of rats with one-kidney, DOCA, salt hypertension. Pullan et al. (1980) measured plasma vasopressin levels and arterial blood pressure in unilaterally nephrectomized dogs before and during renal artery constriction. After six days of renal artery constriction, mean arterial blood pressure was significantly elevated while plasma vasopressin concentration had not significantly changed. Furthermore, when plasma vasopressin concentration was increased five-fold by intravenously infusing vasopressin into normal dogs, there was only a 10 mmHg increase in mean arterial blood pressure. Lee-Kwon et al. (1981) measured plasma vasopressin levels and investigated the effect of intravenous vasopressin antagonist infusion on mean arterial blood pressure in rats with subtotal nephrectomy (70 percent renal mass removed) -salt hypertension. Following subtotal nephrectomy the rats drank tap water for two days and were then given one percent saline for drinking. Plasma vasopressin concentration was elevated 2.5-fold two days and six days after ingestion of saline began. Two days after the substitution of saline for drinking water, the intravenous injection of vasopressin antagonists

caused only a slight reduction in mean arterial blood pressure. This indicates that vasopressin is not the major factor (as a direct pressor agent) in the development and maintenance of partial nephrectomy-salt hypertension.

An increased level of plasma catecholamines also could not fully account for the elevated blood pressure of chronic hypertension. Although intravenous infusion of norepinephrine does increase total peripheral resistance and therefore blood pressure, the effect is transient. Additionally, Kopin et al. (1980) in a literature review of studies on plasma catecholamine concentrations of normotensive and hypertensive patients, found that basal plasma norepinephrine levels are the same in both groups of patients where age-matched normotensive and hypertensive subjects were compared. However, DeChamplain et al. (1981) have reported elevated circulating norepinephrine and epinephrine levels (supine and standing) in a significant number of labile and sustained essential hypertensive patients. Age-matched normotensive and hypertensive patients were compared in this study. DeChamplain et al. postulated that a subgroup of up to 30 percent of essential hypertensive patients may have elevated circulating catecholamines.

Several other studies have shown that catecholamines, via the sympathetic nervous system (central and/or peripheral) may play a role in the development and maintenance of several low renin models of experimental hypertension.

Haeusler et al. (1972) investigated the effects of injections of 6-hydroxydopamine into the lateral ventricle of the brain on the development and maintenance of one-kidney, one clip and one-kidney, DOCA, salt hypertension in rats. In one series of experiments seven-week-old

male Wistar rats were unilaterally nephrectomized and one week later received subcutaneous implants of silastic containing deoxycorticosterone (DOCA). At the same time a 0.9 percent sodium chloride solution was substituted for drinking water. Systolic blood pressure of these rats rose from 126 mmHg before DOCA implant to 215 mmHg six weeks after DOCA implant. Each rat then received three intraventricular injections of 6-hydroxydopamine (250 mg each, dissolved in 0.9 percent w/v NaCl solution containing 1 mg/ml ascorbic acid) at two-day intervals. Systolic blood pressure did not significantly change over the next two weeks. Two weeks after the last injection of 6-hydroxydopamine brain norepinephrine content was decreased to 20 percent, 40 percent, and 12 percent of normal in the hypothalamus, medulla oblongata, and residual parts of the brain, respectively. This procedure was then repeated in another group of rats that were made hypertensive by removing one-kidney and placing a constricting clip on the renal artery of the contralateral kidney. Again, blood pressure was not significantly affected by the intraventricular injection of 6-hydroxydopamine.

In another series of experiments Haeusler et al. gave two to three injections of 6-hydroxydopamine (250 ug each) into the lateral ventricle of seven-week-old male Wistar rats and then tried to induce either one-kidney, one clip or one-kidney, DOCA, salt hypertension in these rats. None of these rats developed hypertension. Systolic blood pressure was 120 mmHg six weeks after renal surgery.

Haeusler et al. concluded that a central "trigger" mechanism which is under the control of norepinephrine and/or dopamine may be responsible for the initiation of one-kidney, one clip and one-kidney, DOCA, salt hypertension in rats.

However, since intraventricular injections of 6-hydroxydopamine did not affect established hypertensions in these models, some other mechanism must be involved in the maintenance of the elevated blood pressure and the "trigger" mechanism must only be involved in the initiation of the hypertension.

Finch and Leach (1970) investigated the effect of intravenous injections of 6-hydroxydopamine on the development and maintenance of one-kidney, one clip and one-kidney, DOCA, salt hypertension in rats. In one series of experiments male rats weighing 130 to 140 g received two intravenous injections of 6-hydroxydopamine (50 mg/kg each, dissolved in 0.001 N HCl) on day one, followed by two intravenous injections of 6-hydroxydopamine (100 mg each) on day seven. On days eight to ten the rats underwent either unilateral nephrectomy with placement of a constricting clip on the contralateral renal artery, or unilateral nephrectomy with subcutaneous implanting of deoxycorticosterone and one percent saline substituted for drinking water. Intravenous injections of 6-hydroxydopamine failed to prevent the development of either one-kidney, one clip or one-kidney, DOCA, salt hypertension in these rats.

In another series of experiments Finch and Leach investigated the effect of intravenously injected 6-hydroxydopamine on the level of arterial blood pressure in rats with established one-kidney, one clip or one-kidney, DOCA, salt hypertension. The 6-hydroxydopamine treatment was the same as in their first series of experiments. Intravenous injection of 6-hydroxydopamine lowered systolic blood pressure of one-kidney, DOCA, salt hypertensive rats from 200 mmHg to 140 mmHg, but in 14 days the systolic blood pressure had returned to 200 mmHg. Intravenous injections of 6-hydroxydopamine lowered the systolic blood pressure

of one-kidney, one clip hypertensive rats from 200 mmHg to 145 mmHg, but the blood pressure gradually returned to its original level over the next five weeks.

Finch and Leach concluded that both one-kidney, one clip and one-kidney, DOCA, salt hypertension can develop and be maintained even though the majority of the sympathetic nervous system has been destroyed. They also speculated that an undetected hormonal system may exist and be responsible for the maintenance of the hypertension.

Douglas et al. (1976) further showed that one-kidney, one clip hypertension in rats can develop when the peripheral sympathetic nervous system has been ablated by adrenal demedullation and chronic guanethidine treatment.

These studies indicate that the role of the sympathetic nervous system and catecholamines in some low renin models of experimental hypertension is uncertain. It appears that central catecholaminergic mechanisms may be more directly involved than the peripheral sympathetic nervous system in the regulation of blood pressure in some models of experimental hypertension. The mechanism of these catecholaminergic pathways in blood pressure control is however unclear.

Furthermore, in addition to an inability to consistently document increased blood levels of the above mentioned vasoconstrictor agents in hypertension, injection of these substances has only a transient effect on blood pressure and does not always increase cardiac contractility (e.g., vasopressin).

These data indicate that there is good reason for seeking another humoral factor which may be responsible for the hemodynamic changes seen in volume-dependent models of hypertension. One possible agent

which has recently gained prominence is a circulating sodium transport inhibitor.

Overbeck and Haddy (1967) measured changes in forelimb blood flow and vascular resistance in the pump-perfused dog forelimb. Measurements were made during the intra-arterial infusion of a potassium chloride solution (1.2 and 2.0 ml/minute) before and nine weeks after the development of one-kidney, one wrapped hypertension. Hypertensive dogs showed a significantly attenuated vasodilator response to infusion of the potassium chloride solutions.

Overbeck and Clark (1975) observed that the vasodilator response to intra-arterially infused potassium chloride solutions (3.07×10^{-3} or 4.60×10^{-3} mEq K^+/minute) into the isolated pump-perfused hindlimb vascular bed of two-kidney, one clip hypertensive rats was significantly reduced compared to control normotensive rats.

Also, Overbeck et al. (1974) have shown that the vasodilator response to intrabrachial artery infusions of an isosmolar potassium chloride solution (0.307 mEq K^+/minute) is attenuated in a proportion of essential hypertensive patients.

Since potassium is thought to cause vasodilation by stimulating the electrogenic Na^+-K^+ pump (Chen et al.,1972), the attenuated responses to potassium seen in several models of hypertension indicate a defect of the vascular Na^+-K^+ pump activity in these animals.

Several studies have been undertaken to determine whether infact there is a defect of cardiovascular muscle cell Na^+-K^+ pump activity in several models of hypertension.

Overbeck et al. (1976) measured ouabain-sensitive ^{86}Rb uptake (a measure of Na^+-K^+ pump activity) in mesenteric arteries and veins

from dogs with one-kidney, one wrapped hypertension and in their paired one-kidney, sham wrapped controls. Ouabain-sensitive ^{86}Rb uptake was depressed by 42 percent and 49 percent in arteries and veins, respectively, from the hypertensive dogs compared to blood vessels from the control dogs.

Pamnani et al. (1978; 1980) showed that vascular Na^+-K^+ pump activity (measured by ouabain-sensitive ^{86}Rb uptake) is significantly depressed in tail arteries from rats with either one-kidney, DOCA, salt or one-kidney, one clip hypertension compared to their respective controls.

Clough et al. (1977a, 1977b) measured Na^+-K^+ ATPase activity in microsomes prepared from the left and right ventricles of one-kidney, one clip hypertensive and paired normotensive rats. Na^+-K^+-ATPase activity was significantly supressed in microsomes prepared from the hypertensive rats compared to those prepared from the normotensive controls.

Because cardiovascular muscle cell Na^+-K^+ pump activity has been found to be suppressed in veins (Overbeck et al.,1976) and right ventricle (Clough et al., 1977b) of hypertensive animals, this indicates that the observed pump supression is not secondary to increased blood pressure.

Furthermore, Pamnani et al. (1980) showed that supernates of boiled plasma from one-kidney, one wrapped hypertensive dogs significantly decrease ouabain-sensitive ^{86}Rb uptake when applied to normal rat tail arteries. This finding indicates that the observed pump suppression may be due to the action of a circulating agent.

Since the Na^+-K^+ pump in cardiovascular muscle cells is electrogenic (Thomas, 1972; Anderson, 1976), suppressed vascular Na^+-K^+ pump

activity could, via voltage-dependent calcium channels (Hendricks and Casteels, 1974) or altered Na/Ca exchange mechanism (Blaustein, 1977), lead to vasoconstriction, increased blood vessel responsiveness to vasoactive agents, and increased cardiac contractility -- changes not unlike those seen in hypertension.

These data indicate that altered cardiovascular muscle cell Na^+-K^+ pump activity may play a role in the genesis and/or maintenance of some low renin, presumably volume-expanded models of hypertension, and that this altered pump activity may be due to a circulating agent.

EFFECTS OF SUPPRESSED CARDIOVASCULAR MUSCLE CELL Na^+-K^+ PUMP ACTIVITY ON HEMODYNAMICS

Suppression of cardiovascular muscle cell Na^+-K^+ pump activity has been shown to increase cardiac contractility (Brace et al., 1974), constrict blood vessels (Vatner et al.,1971; Haddy and Scott, 1973; DeMots et al.,1978), and increase blood vessel responsiveness to vasoactive agents (Brender et al.,1969). Cardiac glycosides such as ouabain, strophanthidin, and acetylstrophanthidin are potent inhibitors of the Na^+-K^+ pump (Glynn, 1964) and have been used by several investigators to study the hemodynamic consequences of induced suppression of cardiovascular muscle cell Na^+-K^+ pump activity.

Brace et al. (1974) measured the effect of acute hypokalemia and/or ouabain on myocardial contractile force and coronary vascular resistance in sodium pentobarbital anesthetized dogs. Two series of experiments were done. In the first series the left common coronary artery was perfused with constant-flow while in the second series the left common coronary artery was perfused with constant-pressure. During constant-flow perfusion and during constant-pressure perfusion of the left

coronary artery, acute hypokalemia caused increased ventricular contractile force and increased coronary vascular resistance. Also, during constant-flow perfusion ouabain (12 ug/minute for 15 minutes) increased ventricular contractile force and coronary vascular resistance. Following the infusion of ouabain, hypokalemia had only small effects on coronary vascular resistance and changes in ventricular contractile force were reduced.

Vatner et al. (1971) investigated the effect of intravenously infused ouabain (0.02 mg/kg) on systemic arterial blood pressure and total peripheral resistance. Five to ten minutes after infusion of ouabain, mean arterial blood pressure increased in conscious dogs (24 ± 3 percent above control) as well as in unconscious dogs (17 ± 2 percent above control). Arterial blood pressure then gradually declined but remained above control for 30 minutes. Ouabain infusion also increased systemic vascular resistance in all dogs, having a greater effect in the conscious dogs. The vascular resistance reached a maximum of 38 ± 5 percent above control three minutes after ouabain infusion in the conscious dogs and remained elevated for 30 minutes.

Haddy and Scott (1973) studied the effect of intravenously infused strophanthidin (50 ug/kg) on arterial blood pressure and total peripheral resistance in sodium pentobarbital anesthetized dogs. Urine flow was monitored and isotonic Ringer's solution was given intravenously at the rate of the urine flow such that extracellular fluid volume did not change. Infusion of strophanthidin increased arterial blood pressure and total peripheral resistance.

DeMots et al. (1978) studied the effects of intravenous ouabain infusion (15 ug/kg) on arterial blood pressure and total peripheral re-

sistance in humans. Ouabain infusion increased mean arterial blood pressure and increased systemic vascular resistance.

Brender et al. (1969) investigated the effects of acetylstrophanthidin (10 ug/ml) on the in vitro contractility of helical strips of dog saphenous vein. Acetylstrophanthidin increased tension and potentiated the contractile response of the strips to electrical stimulation (10 v, 5 cps) and to exogenous norepinephrine (0.1 ug/ml). Also, injection of 0.25 mg of digoxin into the right atria of dogs potentiated the saphenous venomotor response to lumbar sympathetic stimulation.

These studies show that induced suppression of cardiovascular muscle cell Na^+-K^+ pump activity increases cardiac contractility, constricts coronary blood vessels, increases mean arterial blood pressure, constricts systemic blood vessels, and increases blood vessel responsiveness to vasoactive agents. These hemodynamic changes are similar to those seen in several experimental models of hypertension.

A humoral Na^+-K^+ pump inhibiting agent could also affect the hemodynamics of hypertensive animals by altering norepinephrine uptake at sympathetic nerve terminals. The uptake or re-uptake of norepinephrine across the neuronal membrane, followed by binding and storage of the catecholamine by specific granules, is the major means of inactivating circulating catecholamines (Axelrod, 1965).

DeChamplain et al. (1969) have shown that rats with one-kidney, DOCA, salt hypertension have a reduction in the ability to store norepinenephrine and this occurs prior to development of hypertension. Also, the storage of cardiac norepinephrine was negatively correlated with the level of dietary sodium intake in one-kidney, DOCA, salt hypertensive rats (DeChamplain et al., 1969).

LeLorier et al. (1976) showed that the capacity of the sympathetic nerve endings to take up norepinephrine in heart and aorta of rats with "adrenal regeneration" (unilateral nephrectomy with unilateral adrenalectomy, contralateral adrenal enucleation, and one percent NaCl solution for drinking), one-kidney, Grollman wrap (with one percent NaCl solution for drinking), and sodium-loaded (ten percent NaCl solution for drinking) hypertension is decreased compared to their respective controls. Furthermore, in the one-kidney, Grollman wrap hypertensive rats, there was no difference in the time of appearance of hypertension and the decrease of norepinephrine uptake by the heart or aorta.

Nakazato et al., (1978) showed that ouabain (10^{-4}M) causes an increase in the release of norepinephrine by isolated vas deferentia of guinea pigs. They suggested that this might be due to an effect of ouabain on the Na^{+}-dependent Ca^{2+} influx system.

Increased norepinephrine release and/or decreased norepinephrine re-uptake at sympathetic nerve endings would result in increased levels of norepinephrine at the synaptic cleft. Increased synaptic norepinephrine levels would enhance vasoconstriction and increase cardiac contractility.

If norepinephrine release is, however, increased and re-uptake is decreased over a long period of time, tissue catecholamine levels would eventual decrease. Decreased tissue catecholamine levels have been reported following prolonged administration of the cardiac glycoside digitalis (Gillis and Quest, 1979) and in rats with one-kidney, DOCA, salt hypertension (DeChamplain et al., 1969). One possible side effect of these events is denervation supersensitivity which would enhance vasoconstrictor responses to circulating catecholamines

(Trendelenburg, 1963; 1966)

These studies indicate that a circulating Na^+-K^+ pump inhibiting factor could cause hemodymanic changes similar to those seen in hypertension by directly affecting muscle cells and by affecting norepinephrine uptake at sympathetic nerve endings.

POSSIBLE SOURCE OF THE CIRCULATING SODIUM TRANSPORT INHIBITING FACTOR

Since the development of some forms of experimental hypertension can be prevented by destruction of central catecholamine containing neurons (Haeusler et al., 1972), much consideration has been given to the site and mechanism of action of centrally administered catecholamine destroying agents (e.g. 6-hydroxydopamine). Several investigators have shown that ablation of the anteroventral third ventricle (AV3V) region of the brain prevents the development of several low renin models of experimental hypertension (Fink et al., 1977; Buggy et al.,1977; Brody et al., 1978).

The AV3V region encompasses the immediate periventricular tissue in the most anterior and ventral portion of the third ventricle (Brody et al, 1980), and has been shown to contain angiotensin receptors which mediate drinking and pressor activity (Buggy and Johnson, 1977). Furthermore, in rats, this region of the brain lacks a blood-brain barrier and could therefore play an important role in the homeostasis of blood pressure and body fluid volumes.

Fink et al. (1977) studied the effect of electrolytic lesions of the AV3V region on the development of one-kidney, DOCA, salt hypertension in rats. Male Sprague-Dawley rats underwent AV3V lesioning or sham-lesioning procedures and all rats subsequently received weekly subcutaneous injections of deoxycorticosterone (14 mg) while one percent

sodium chloride solution was substituted for drinking water. The sham lesioned rats became hypertensive (systolic blood pressure 150 mmHg) within three weeks of the DOCA, salt treatment and their systolic blood pressure leveled off at 170 mm Hg at five weeks. In AV3V lesioned rats, DOCA, salt treatment produced no significant increase in arterial blood pressure. Fink et al. concluded that the AV3V region contains structures necessary for the development of one-kidney, DOCA, salt hypertension in rats.

Buggy et al. (1977) investigated the effect of electrolytic lesions of the AV3V region on the development of one-kidney, Grollman wrap (figure of eight ligature around the parenchyma of one kidney with contralateral nephrectomy) hypertension. Male Sprague-Dawley rats were divided into two groups. Group I rats underwent elecrolytic lesioning of the AV3V region while group II rats underwent a sham-lesioning procedure. One half of each of these groups of rats were then unilaterally nephrectomized and a figure eight ligature placed around the contralateral kidney while the other half of the groups underwent sham operating procedures. Sham-lesioned and AV3V lesioned rats that did not undergo the one-kidney, Grollman wrap surgery remained normotensive throughout the three-week period of the study. Sham-lesioned rats with one-kidney, renal wrap surgery showed significantly increased fluid intake and became hypertensive (systolic blood pressure 175 mmHg 10 days after renal surgery). Rats with AV3V lesions and one-kidney renal wrapping did not show an increase in fluid intake and they did not become hypertensive (systolic blood pressure 135 mmHg 10 days after renal surgery). Buggy et al. concluded that the AV3V region of the brain contains structures which play a role in the development

of one-kidney, Grollman wrap hypertension in rats.

The anatomical structures commonly destroyed by the AV3V lesion include preoptic periventricular nuclei, anterior hypothalamic periventricular nuclei, the median preoptic nucleus, and the anterior wall of the third ventricle with the associated organum vasculosum of the lamina terminalis (Brody et al, 1978). In addition to the effect of the AV3V region on the development of some models of experimental hypertension, this region has also been shown to be involved as a dipsogenic site for the action of hyperosmotic and angiotensin thirst stimuli (Buggy and Johnson, 1977). Bealer et al. (1979) have shown that lesions of the AV3V region prevents the release of sodium transport inhibiting factor into the plasma of volume expanded rats. Also, Pamanani et al. (1981) have shown that lesions of the AV3V region prevent the appearance of a circulating ouabain-like factor in the plasma of volume expanded rats.

Together, these studies indicate that the AV3V region of the brain is involved in the development of some models of experimental hypertension and in the release and/or synthesis of Na^+ transport inhibiting factor(s) in response to volume expansion. These data also indicate that prevention of the release of the Na^+ transport inhibiting factor may be the mechanism by which the AV3V region prevents the development of some models of experimental hypertension.

EXPERIMENTAL MODELS OF LOW RENIN, VOLUME-DEPENDENT HYPERTENSION

There are several models of low renin, volume-dependent hypertension which can be studied. These include one-kidney, one clip (unilateral nephrectomy with constricting clip on renal artery of contralateral kidney) one-kidney, DOCA, salt (unilateral nephrectomy followed by

weekly subcutaneous injections of deoxycorticosterone while providing one percent saline for drinking) and one-kidney, one wrapped (unilateral nephrectomy with silk bag around contralateral kidney) hypertension. Although all of these models have been documented to have low or normal plasma renin activity, their volume status is an unresolved issue. Some investigators have reported increased volume while others report no change or decreased volume.

One model of hypertension which has been documented to be low renin and volume expanded is the reduced renal mass model (70 to 80 percent renal mass removed). Ylitalo and Gross (1979) reported that following subtotal nephrectomy (75 percent renal mass removed) rats consuming a high sodium chow (750 mEq/kg) become hypertensive (systolic blood pressure > 140 mmHg). When studied four weeks later hypertensive animals had significantly suppressed plasma renin activity, increased absolute (ml) and relative (ml/kg body weight) extracellular fluid volume, and increased relative plasma volume (ml/kg body weight). Pitcock et al. (1980) have also reported that rats with reduced renal mass hypertension have increased absolute and relative extracellular fluid volumes.

The reduced renal mass model of hypertnesion is the model I have chosen to study for my thesis.

WORK BY OTHERS WITH THE REDUCED RENAL MASS MODEL OF HYPERTENSION

Schmidt and co-workers (1974) prepared reduced renal mass (70 to 80 percent renal mass removed) female, mongrel dogs and divided them into control and experimental groups. Experimental animals were maintained on a constant sodium intake (120 mEq sodium per day) while control animals had their sodium intake reduced in direct proportion to

their reduction in glomerular filtration rate following subtotal nephrectomy. In the experimental group fractional excretion of sodium per single nephron increased from 0.30 percent of the filtered sodium before nephrectomy to 4.50 percent of the filtered sodium after surgery, whereas in the control group fractional excretion of sodium per single nephron did not change after nephrectomy. A fraction prepared by gel filtration of serum from experimental dogs caused natriuresis when injected into assay rats. A similar fraction obtained from the serum of control dogs did not affect natriuresis. Schmidt et al. concluded that the adaptive mechanisms which maintain external sodium balance when the renal mass is reduced are associated with an increased fractional excretion of sodium per single nephron and the appearance of a circulating natriuretic factor which mediates this response.

This group did not report arterial blood pressure of the animals and they also did not test this serum fraction on cardiovascular tissue.

Espinel (1975) investigated the effects of reduction of renal mass on the fractional excretion of sodium per single nephron in rats. After surgically removing 70 to 80 percent of each animal's renal mass, the rats were divided into control and experimental groups. In the experimental group dietary sodium intake was reduced in direct proportion to the reduction in glomerular filtration rate. In the control group dietary sodium intake was unrestricted. Fractional excretion of sodium per single nephron for the control rats increased from 0.32 percent of the filtered sodium before nephrectomy to 1.83 percent of the filtered sodium after reduced renal mass surgery. Fractional excretion of sodium per single nephron did not change in the experimental group.

External sodium balance was maintained by all rats. Espinel concluded that in chronic uremia there are "adaptive mechanisms" which increase sodium excretion per single nephron and function to maintain external sodium balance. He did not speculate on what these mechanisms might be.

Espinel (1976) prepared similar groups of reduced renal mass rats and measured changes in arterial blood pressure in addition to changes in the fractional excretion of sodium per single nephron. The control rats (unrestricted dietary sodium intake) became hypertensive (systolic blood pressure 210 ± 15 mmHg) while the experimental rats (reduced sodium intake) did not become hypertensive (systolic blood pressure 150 ± 16 mmHg). Also, fractional excretion of sodium per single nephron increased in the control rats and remained unchanged in the experimental rats. Espinel concluded that in experimental chronic renal failure the adaptive mechanisms developed to maintain external sodium balance on unrestricted dietary sodium intake are associated with the development of hypertension. He did not speculate on what these mechanisms might be. He also did not look for the presence of a circulating sodium transport inhibitor and he did not measure cardiovascular muscle cell Na^+-K^+ pump activity in these rats.

Ylitalo et al. (1976) studied the effects of various sodium intakes on blood pressure and the renin-angiotensin system in rats in which the renal mass had been reduced by 70 percent. Reduced renal mass rats were divided into three groups: group I, high sodium diet (750 mEq Na^+/Kg); group II, standard sodium diet (150 mEq Na^+/Kg); and group III, low sodium diet (less than 0.2 mEq Na^+/Kg). Potassium content was 150 mEq/kg in each diet. Average systolic blood pressure before subtotal nephrectomy was 116 ± 2 mmHg. By 25 days after subtotal nephrec-

tomy, systolic blood pressure of the group I rats had increased to 185 ± 9 mmHg, systolic blood pressure of the group II rats had risen to 131 ± 3 mmHg, and systolic blood pressure of group III rats showed no change. Plasma angiotensin II concentration (pg/ml) for groups I, II, and III were 12 ± 3, 37 ± 4, and 374 ± 23, respectively. Kidney renin concentration (ug angiotensin I/20 minutes/kidney) for groups I, II, and III were 9 ± 1, 74 ± 6, and 220 ± 13, respectively. These investigators concluded that (1) a reduction in renal mass is associated with hypertension when the sodium intake is increased and (2) that the renin-angiotensin system is not responsible for the elevated blood pressure. They also concluded that the elevated blood pressure in group I rats might be explained by Guyton's hypothesis of long term whole-body autoregulation. They did not assay for a circulating sodium transport inhibitor and they did not measure cardiovascular muscle cell Na^+-K^+ pump activity.

Ylitalo and Gross (1979) studied the hemodynamic changes that occur during the development of reduced renal mass-salt hypertension. Three groups of rats were prepared. Groups I and II consisted of sham operated rats consuming a standard or a high sodium diet (150 and 750 mEq/kg), respectively. Group III was made up of rats with subtotal nephrectomy (70 percent renal mass removed) that were fed the high sodium diet. Systolic blood pressure, plasma volumes, and extracellular fluid volumes were monitored for four weeks. Plasma renin activity was measured at the end of the fourth week. Systolic blood pressure continuously increased in group III rats, reaching 178 ± 9 mmHg by the fourth week. Blood pressure of all other rats did not change. Absolute plasma volume was not significantly different in any of the groups; however,

relative plasma volume was 25 percent higher in group III rats than in group I or group II rats by the third and fourth week. Relative extracellular fluid volume was increased in the group III rats by the seventh post-operative day and was 43 percent greater in these rats than in the group I or group II rats by the fourth week. By the fourth week absolute extracellular fluid volume was increased in group III rats compared to the sham-operated rats. Plasma renin activity of group III rats was suppressed to 20 percent of that in sham-operated animals on the high sodium diet. Plasma renin activities of group I and group II rats were not significantly different. Ylitalo and Gross concluded that expansion of the extracellular and intravascular spaces resulted in the development of hypertension. They did not speculate on the mechanism of this hypertension nor did they assay for a circulating sodium transport inhibitor or measure cardiovascular muscle cell Na^+-K^+ pump activity.

PURPOSE OF THE INVESTIGATION

Data from our own and other laboratories suggest a possible role of cardiovascular muscle cell Na^+-K^+ pump activity in the development and maintenance of some low renin models of experimental hypertension. However, the role of body fluid volumes, the influence of the sympathetic nervous system, and the mechanism by which cardiovascular muscle cell Na^+-K^+ pump activity is inhibited remain obscure.

The objectives of this investigation were to: 1) measure vascular Na^+-K^+ pump and microsomal Na^+,K^+-ATPase activities in tail arteries and myocardium, respectively, from control normotensive and experimental hypertensive reduced renal mass rats; 2) determine if the depressed pump activity (if present) is due to a circulating ouabain-like agent; 3) determine if there is a temporal correlation between suppression of vascular Na^+-K^+ pump activity and the development of reduced renal mass hypertension; 4) determine the role of the peripheral sympathetic nervous system in the development of reduced renal mass hypertension and in vascular Na^+-K^+ pump activity; 5) determine the role of the central sympathetic nervous system in the development of reduced renal mass hypertension, vascular Na^+- K^+ pump activity, and the appearance of ouabain-like factor in plasma; 6) determine the effect of local sympathetic nerve endings on Na^+- K^+ pump activity in control normotensive and experimental hypertensive reduced renal mass rats; 7) determine the role of the anteroventral third ventricle (AV3V) region of the brain in the development of reduced renal mass hypertension, vascular Na^+-K^+ pump activity, and appearance of ouabain-like factor in plasma; 8) measure plasma renin activity of control normotensive and experimental hyperten-

sive reduced renal mass rats; and 9) measure absolute (ml) and relative (ml/kg body weight) plasma and extracellular fluid volumes of control normotensive and experimental hypertensive reduced renal mass rats.

MATERIALS AND METHODS

Na^+-K^+ PUMP ACTIVITY IN CARDIOVASCULAR MUSCLE CELLS OF REDUCED RENAL MASS RATS

Preparation of the Reduced Renal Mass Model of Hypertension

Male Wistar rats (Charles River) weighing 200-225 g were used throughout this study. Initially normotension was documented in all rats by measurement of systolic blood pressures (tail plethysmography, Natume, KN-209) in the conscious state. Rats having systolic blood pressures less than 130 mmHg on two separate weekly readings were used. These rats were randomly divided into pairs, each pair consisting of a control and an experimental rat and all animals then underwent subtotal nephrectomy. Under light ether anesthesia, through a midline abdominal incission, the right kidney and approximately 50 percent of the left (both poles) were removed. The removal of 50 percent of the left kidney was estimated with the assumption that both kidneys were equal in weight. Silk ties were used to excise the poles of the left kidney. A loop of 4-0 silk suture was placed around each pole of the kidney and then pulled tight. This both cut the tissue and tied off the main arterial and venous supply of the excised areas. All rats then consumed a low sodium chow (0.02 percent sodium, 0.59 percent potassium, Bioservices Inc.) and control rats drank distilled water while experimental animals drank a one percent NaCl solution. Daily sodium intake was monitored in some control and experimental rats which were individually housed in metabolic cages. Systolic blood pressure (tail plethysmography, Natume-KN209) and body weights were monitored weekly. Animals were considered hypertensive when their systolic blood pressure was

greater than 140 mmHg. In some experimental and control rats direct blood pressures were recorded in the conscious state via indwelling carotid artery cannulas. Under light ether anesthesia a cannula (PE60, Clay Adams Co.) filled with saline containing heparin (100 units/ml Porcine Mucosal, Lypho-Med, Inc.) was placed in the right common carotid artery. The cannula was then passed under the skin and exteriorized through a small incision behind the neck. Next, the cannula was connected to a P 23-GB transducer (Gould-Statham Inc.) which was connected to a Hewlett-Packard recorder (Model 7758B). Systolic, diastolic and mean arterial blood pressures were then recorded.

Measurement of Na^+-K^+ Pump Activity

Two methods were used to measure cardiovascular muscle cell Na^+-K^+ pump activity: 1) for vascular smooth muscle cells ouabain-sensitive radioactive rubidium uptake (initial velocity) by blood vessels was used and 2) for myocardial cells microsommal Na^+, K^+-ATPase activity was used.

After four weeks of sustained hypertension in the experimental (saline drinking) reduced renal mass rats and after a similar time period in the paired control (distilled water drinking) reduced renal mass rats, vascular Na^+-K^+ pump activity and microsomal Na^+, K^+-ATPase activity were measured in tail arteries and myocardium, respectively.

Under sodium pentobarbital anesthesia (75 mg/kg ip), ventral tail arteries and hearts were removed from control (distilled water drinking) and experimental (saline drinking) reduced renal mass rats. The tail arteries were placed in a Krebs-Henseleit solution (27.2 mM $NaHCO_3$, 118.0 mM NaCl, 4.8 mM KCL, 1.0 mM KH_2PO_4, 1.2 mM $MgSO_4 \cdot 7H_2O$, 11.1 mM glucose, and 2.5 mM $CaCl_2 \cdot 2H_2O$), aerated with a 95 percent

O_2, 5 percent CO_2 mixture and allowed to equilibrate for 10 minutes. The hearts were immediately placed in a cold solution containing 5 mM ethylenediamine tetraacetic acid (EDTA), 0.25 M sucrose, and 5 mM histidine-HCL (pH 7.4).

Radioactive Rubidium Uptake by Blood Vessels. Ouabain-sensitive ^{86}Rb (New England Nuclear) uptake was used to measure vascular smooth muscle cell Na^+-K^+ pump activity. This technique is standard and has been used by others to estimate Na^+-K^+ pump activity in myocardium (Ku et al., 1974), erythrocytes, and brain tissues (Bernstein and Israel, 1970). In our laboratory (Overbeck et al., 1976; Pamnani et al., 1978; 1981) this technique has been used to measure vascular Na^+-K^+ pump activity.

There are several monovalent cations which can substitute for K^+ and be actively transported by the Na^+-K^+ pump. These cations, in order of decreasing apparent affinity, are Tl^+, Rb^+, NH^+, and Li^+ (Dahl and Hokin, 1974; Robinson, 1979). Although ^{42}K has been used to study K fluxes (Hilton and Patrick, 1973) ^{86}Rb has advantages over ^{42}K in the measurement of Na^+-K^+ pump activity. ^{86}Rb has a longer half-life and a lower energy emission than the radioactive form of potassium. In addition to substituting for potassium in active transport by the Na^+-K^+ pump, rubidium also has the same physiological actions of potassium (eg. rubidium vasodilation has been shown to be indistinguishable from potassium vasodilation in the coronary vascular bed of guinea pig heart, Bunger et al., 1976). The aim of the rubidium uptake technique was to initially slow the pump (to load the cells with sodium) and to then stimulate the pump and measure its activity. We measured pump activity under these stimulated conditions because we felt that this would

most likely reveal a pump defect if one were present. A defect too small to be measurable under steady-state conditions (but large enough to have important physiological effects over a long period of time) might be exaggerated sufficiently to be measurable under stimulated conditions. Total ^{86}Rb uptake and ouabain-insensitive ^{86}Rb uptake were measured in each vessel. The difference between these two values is ouabain-sensitive ^{86}Rb uptake. Ouabain-sensitive ^{86}Rb uptake is a measure of Na^+-K^+ pump activity, whereas ouabain-insensitive ^{86}Rb uptake reflects distribution in extracellular space and passive penetration into cells which depends upon permeability, surface area, and the concentration gradient. The detailed method was as follows.

The tail arteries excised from control and experimental reduced renal mass rats were dissected free of adventitia, opened longitudinally, cut into 2 cm long pieces, randomly divided into two groups (each group consisting of one half of the tail artery) and placed in a 0°C K^+-free Krebs-Henseleit solution (27.2 mM $NaHCO_3$, 117.0 mM NaCl, 1.0 mM $NaH_2PO_4 \cdot H_2O$, 1.2 mM $MgSO_4 \cdot 7H_2O$, 11.1 mM glucose, and 2.5 mM $CaCl_2$) for 10 minutes to depress the pump and load the cells with sodium (Thomas, 1972). One half of each artery was then incubated at 37°C in a K^+-free Krebs-Henseleit solution containing 2 mM "cold" rubidium chloride. The other half of each artery was incubated in a K^+-free Krebs-Henseleit solution containing 0.8 mM ouabain. A standard concentration of ^{86}Rb was then added to each medium (0.01 to 0.03 mM depending on the specific activity) and the incubation continued for 18 minutes. The media were aerated with 95 percent O_2, 5 percent CO_2 during the incubation. The tissues were then removed from the incubation medium, rapidly washed three times with a potassium-free Krebs-Henseleit solution containing

2 mM rubidium chloride (to stop further uptake of ^{86}Rb), blotted with tissue paper to remove the surface fluid, and placed in a scintillation counter (Searle 1185) to measure ^{86}Rb uptake. The tissues were then placed in an oven at 100°C for 24 hours and dry weight of the tissues determined. Rubidium 86 uptake was expressed as pMoles/mg tissue dry weight. Ouabain-sensitive uptake was calculated as the difference between ^{86}Rb uptake without and with ouabain.

Since initial experiments indicated that compared to tail arteries from normotensive controls, ouabain-sensitive ^{86}Rb uptake was depressed in tail arteries from experimental hypertensive rats, ouabain and RbCl dose response curves were generated for tail arteries from control and experimental reduced renal mass rats to determine whether the observed difference in Na^+-K^+ pump activity was due to a difference in sensitivity to inhibition by ouabain and/or Rb affinity. The method for the ouabain dose response curve was as follows.

Tail arteries were excised from sodium pentobarbital anesthetized control normotensive and paired experimental hypertensive reduced renal mass rats, and placed in a 0°C K^+-free Krebs-Henseleit solution for 10 minutes as previously described. Two pieces of each artery were then incubated at 37°C in a K^+-free Krebs-Henseleit solution containing 2 mM RbCl plus a known concentration of ouabain (0.0, 0.05, 0.10, 0.20, 0.40, 0.60, or 0.80 mM), ^{86}Rb was then added, the incubation continued for 18 minutes, and ^{86}Rb uptake calculated as pMoles/mg tissue dry weight as previously described.

The method for determining the apparent k_m for Rb was as follows. Tail arteries were removed from sodium pentobarbital anesthetized control normotensive and experimental hypertensive reduced renal mass

rats and placed in a K^+-free Krebs-Henseleit solution at 0°C as previously described. Two pieces of each artery were then incubated at 37°C in a K^+-free Krebs-Henseleit solution containing known amounts of RbCl (0.5, 1.0, 2.0, 4.0, 6.0, or 8.0 mM), ^{86}Rb added, the incubation continued for 18 minutes, and ^{86}Rb uptake calculated as pMoles/mg tissue dry weight as previously described.

Na$^+$, K$^+$-ATPase Activity of Cardiac Microsomes. Membrane-bound Na^+, K^+-ATPase is the enzyme which provides energy for the active transport of sodium and potassium across cell membranes (Wallick, et al., 1979). Wallick et al. (1979) have shown that ouabain-inhibitable, ATP-dependent Na^+-K^+ transport can be measured in vesicles reconstituted from purified Na^+, K^+-ATPase and phospholipids, thus providing direct evidence that Na^+, K^+-ATPase is in fact the Na^+-K^+ pump. Like Na^+-K^+ pump activity in intact cells, the ability of isolated membranes to split ATP, (i.e. the total ATPase activity) is inhibited by the addition of ouabain to and by the removal of potassium from the incubation medium. Any residual activity is due to Mg^{2+}-ATPase. Na^+, K^+-ATPase activity is then the difference between total ATPase and Mg^{2+}-ATPase activities. Since it is technically difficult to measure Na^+, K^+-ATPase activity in membrane preparations of vascular smooth muscle (Wei et al., 1976; Wei et al., 1977), microsomes isolated from the left ventricle of control and experimental reduced renal mass rats were used to measure Na^+, K^+-ATPase activity in this study. Microsomal fractions were prepared by a method similar to that of Akera, et al. (1969). Briefly, the method was as follows.

The left ventricle and interventricular septum were separated from the myocardium of control and experimental reduced renal mass rats,

minced with a scalpel, and placed in 10 ml of a cold solution containing 0.15 percent deoxycholate, 5 mM EDTA, 0.25 M sucrose, and 5 mM histidine adjusted with tris base to pH 6.8. This tissue was then kept on ice and homogenized by ten strokes with a motor-driven pestel (Brinkman Instruments). The homogenate was then centrifuged in a Sorvall RC-5 refrigerated centrifuge (SM-24 rotor) at 1,100 X g for 10 minutes to remove the nuclear fraction, and then at 12,300 X g for 20 minutes to remove the mitochondrial fraction. The supernate was then collected and centrifuged (Sorvall RC-5; SM-24 Rotor) at 35,600 X g for 90 minutes to obtain the microsomal fraction. The microsomal fraction was rehomogenized in 5 ml of a cold solution containing 5 mM EDTA, 1 M NaI, and 5 mM histidine (pH 6.8) and then placed on ice and mixed for one hour. The microsomal fraction was then recovered by centrifugation (Sorvall RC-5; SM-24 Rotor) at 35,600 X g for 90 minutes, resuspended in cold imidazole-HCl (pH 7.4), and stored overnight on ice in a refrigerator.

Microsomal ATPase activity, maximal velocity, was assayed by measuring the amount of inorganic phosphate (Pi) liberated from ATP (Tris-ATP from equine Muscle, Sigma Chemical Company) during incubation of microsomes for one hour at 37°C in a water bath shaker. The assay medium for total ATPase activity contained in 2 ml: 120 mM NaCl, 10 mM KCl, 40 mM tris-HCl (pH 7.5 at 37°C), 2 mM Tris-ATP, 2.5 mM $MgCl_2$, and 0.5 mM ethyleneglycolbis-(aminoethylether)-N, N^1-tetraacetic acid (EGTA). EGTA was included to chelate any contaminant Ca^{2+} which would inhibit Na^+, K^+-ATPase activity. Mg^{2+}-ATPase activity was assayed under similar conditions except that 1 mM ouabain replaced KCl in the assay medium. Na^+, K^+-ATPase activity was calculated by subtracting Mg^{2+}-ATPase activity from total ATPase activity. ATPase activities were expressed

as uMoles Pi/mg protein/hour. Inorganic phosphate was assayed by the method of Fiske and SubbaRow (1925) and microsomal protein was assayed by the method of Lowry et al. (1951). Since initial experiments indicated that microsomal Na^+, K^+-ATPase activity was less in myocardium from experimental hypertensive rats compared to normotensive controls, sialic acid content of microsomes prepared from myocardium of control and experimental rats was measured in Dr. Clough's lab using the thiobarbituric acid method of Aminoff (1961) to determine if the observed difference in Na^+, K^+-ATPase activity was due to a difference in the amount of plasma membrane.

TEMPORAL CORRELATION BETWEEN DEPRESSED VASCULAR Na^+-K^+ PUMP ACTIVITY AND THE DEVELOPMENT OF REDUCED RENAL MASS HYPERTENSION

If suppression of cardiovascular muscle cell Na^+-K^+ pump activity of hypertensive rats (as observed in our preliminary experiments) is responsible for the initiation of the elevated blood pressure seen in these animals, there should be a temporal relationship between supression of vascular Na^+-K^+ pump activity and the development of hypertension. Therefore, in these experiments another group of subtotally nephrectomized control (distilled water drinking) and experimental (saline drinking) rats were prepared and their systolic blood pressure monitored weekly for five weeks following subtotal nephrectomy. At weekly intervals some control and experimental rats were anesthetized with sodium pentobarbital and their tail arteries excised for measurement of vascular Na^+-K^+ pump activity as previously described.

ROLE OF THE SYMPATHETIC NERVOUS SYSTEM IN THE DEVELOPMENT OF REDUCED RENAL MASS HYPERTENSION AND VASCULAR Na^+-K^+ PUMP ACTIVITY

One means of assessing the role of the sympathetic nervous sys-

tem in the development of experimental hypertension is to evaluate the effect of sympathectomy on the genesis and maintenance of hypertension. Different results have been obtained with other models of hypertension (Finch and Leach, 1970; Haeusler et al. 1972) depending on whether the peripheral or the central sympathetic nevous system was destroyed.

6-hydroxydopamine causes a selective destruction of adrenergic nerve terminals (Thoenen and Tranzer, 1968; Finch et al., 1973; Sachs and Jonsson, 1975). Electron microscopy studies have shown that 6-hydroxydopamine is taken up by adrenergic nerve endings and results in a degeneration of these nerve endings (Thoenen and Tranzer, 1968; Uretsky and Iversen, 1970). It has been injected intravenously to destroy peripheral sympathetic nerve endings (Thoenen and Tranzer, 1968; Haeusler et al., 1969; Finch and Leach, 1970) and intraventricularly in the brain to deplete central catecholamines (Haeusler et al., 1972; Breese, 1975).

Peripheral Sympathectomy

In another series of experiments the role of the peripheral sympathetic nervous system in the development of reduced renal mass hypertension and vascular Na^+-K^+ pump activity was studied.

A modification of the method of Finch and Leach (1970) was used to produce peripheral sympathectomy. Male Wistar rats (180-200 g) with adrenal medullectomy (Charles River) and with documented normotension were given two intravenous bolus injections, through an external jugular vein cannula, of 6-hydroxydopamine (50 mg/kg each) dissolved in 0.9 percent sterile saline containing 1 mg/ml ascorbic acid to prevent oxidation of the 6-hydroxydopamine. Seven days later all rats received two additional injections, through an external jugular vein cannula, of 6-

hydroxydopamine (100 mg/kg each). Two weeks after the last injection all rats underwent subtotal nephrectomy and were divided into control (distilled water drinking) and experimental (saline drinking) groups as previously described. Systolic blood pressures and body weight were monitored weekly for five weeks. Paired control and experimental rats were then anesthetized with sodium pentobarbital, their tail arteries excised for measurement of ouabain-sensitive and insensitive rubidium 86 uptakes as previously described, and 4 ml of aortic blood collected for measurement of plasma catecholamine levels to evaluate the effectiveness of the sympathectomy. Plasma catecholamines were measured by radioimmunoassay as follows.

An Upjohn diagnostics radioimmunoassay kit was used to measure plasma catecholamine levels. A modification (Upjohn Diagnostics, 1978) of the method of Passon and Peuler (1973) was used. This method measures the level of catecholamines (norepinephrine and epinephrine) in plasma samples. The principle of the assay is as follows: Norepinephrine and epinephrine were simultaniously converted to their corresponding meta-^{3}H-methoxy derivatives by the catalytic action of a partially purified preparation of COMT in the presence of S-adenosyl-L-methionine-[^{3}H-methyl]. Following this enzyme-catalyzed O-methylation of catecholamines, the catecholamine derivatives (^{3}H-normetanephrine and ^{3}H-metanephrine) were then converted by periodate (sodium metaperiodate solution) oxidation to ^{3}H-vanallin which was then extracted. The radioactivity in each extract was then proportional to the amount of norepinephrine and epinephrine in plasma samples.

Four ml of aortic blood were collected from sodium pentobarbital anesthetized, 6-hydroxydopamine-treated control (distilled water drink-

ing) and experimental (saline drinking) reduced renal mass rats and put into evacuated tubes (Upjohn Diagnostics) containing EGTA (7.2 mg) and glutathione (4.8 mg). The blood samples were then centrifuged at 1,100 X g for 10 minutes in a refrigerated centrifuge (Sorvall RC-3B; H-6000 swinging bucket rotor) and the plasma separated and frozen until assayed for catecholamines. On the assay day the plasma samples were thawed and 50 ul of each sample pipetted into another tube. S-adenosyl-L-methionine [^{3}H-methyl] and the enzyme catechol-O-methyl transferase were then added to each sample and all tubes incubated at 37°C for 60 minutes in a shaking water bath. Fifty ul of periodate was then added to each sample to convert the ^{3}H-catecholamine derivatives to ^{3}H-vanallin. These samples were then extracted with 0.1 M acetic acid, centrifuged (Sorvall RC-3B; H-6000 Swinging bucket rotor) and the upper organic phase from each tube collected. Each of these samples were then counted in a beta-scintillation counter (Searle Analytic-81) and compared with a standard for determination of catecholamines. Plasma catecholamine levels were expressed as pg/ml. The standard was prepared by diluting the catecholamine standard solution (containing 100 ug of l-norepinephrine, l-epinephrine, and dopamine per ml in acidic glutathione, contained in the radioimmunoassay kit) 1:10,000 with distilled water to a concentration of 10 ng/ml. The standard for the assay contained 10 ul (100 picograms of each catecholamine) of this diluted solution.

Central Sympathectomy

Because preliminary data indicated that peripheral sympathectomy did not prevent the development of reduced renal mass-saline hypertension, experiments were designed to determine whether central sympathec-

tomy would have different results as reported with other models of experimental hypertension (Haeusler et al., 1972). Therefore, in another series of experiments the role of the central sympathetic nervous system in the development of reduced renal mass hypertension and vascular Na^+-K^+ pump activity was studied. The method of Breese and Howard (1971) was used to produce central sympathectomy.

Male Wistar rats (170 to 190 g) with documented normotension were anesthetized with ether. Using a 20 gauge hypodermic needle attached to a tuberculin syringe, a hole through the skull was made 1 mm lateral of the midsaggital suture and 1 mm posterior of the anterior coronal suture. 6-hydroxydopamine HCl (200 ug) dissolved in 10 ul of 0.9 percent sterile saline containing 1 mg/ml ascorbic acid (to prevent oxidation) was injected into the right lateral ventricle. A 50 ul Hamilton syringe with a protective polyethylene sleeve (which allowed only a 4 mm penetration into the brain) was used to inject the 6-hydroxydopamine. One week later this procedure was repeated, except that the 6-hydroxydopamine was injected into the left lateral ventricle.

Two weeks after the last injection of 6-hydroxydopamine all rats underwent subtotal nephrectomy and were divided into control (distilled water drinking) and experimental (saline drinking) groups as previously described. Systolic blood pressures and body weight were monitored weekly for five weeks. Paired control and experimental rats were then anesthetized with sodium pentobarbital and their tail arteries excised for measurement of ouabain-sensitive and insensitive rubidium 86 uptakes, six ml of aortic blood collected for assay of ouabain-like activity, and brains removed from some of the animals for measurement of brain catecholamine content to determine the success of the sympathecto-

my. The protocol for assay of ouabain-like activity in blood is described later. Brain catecholamine content was measured by Dr. Douglas Petibone according to the electrochemical method of Felice et al. (1978) as modified by Hefti et al. (1980).

For assay of catecholamines, brains were homogenized in 0.1 M perchloric acid containing 0.4 mM sodium bisulfate as an antioxidant. Catecholamines (norepinephrine, dopa, epinephrine and dopamine) were simultaneously assayed using reversed-phase high pressure liquid chromatography (HPLC) combined with electrochemical detection. Catecholamines in the perchloric acid extract were first purified by absorption to alumina microcolumns before injections into the HPLC (Hewlett-Packard 1084 liquid chromatograph). A reversed-phase C-18 Ubondapak column (Waters Associates) was used as the stationary phase; the mobile phase (0.1 M sodium phosphate buffer, pH 3.3, containing 0.5 mM heptane sulfonic acid and 1 mM EDTA) was pumped at 1.6 ml/minute. The catechols were detected using a Bioanalytical systems electrochemical detector set at a potential of $^{+}0.7$ volts. The sensitivity of the assay was 50 to 100 pg for the catecholamines.

Effect of In Vitro Denervation of the Rat Tail Artery on Vascular Na^{+}-K^{+} Pump Activity

Since the rat tail artery is richly innervated with sympathetic nerve endings and since catecholamines stimulate Na^{+},K^{+}-ATPase activity (Limas and Cohn, 1974) we wondered whether the observed decreased vascular Na^{+}-K^{+} pump activity in rats with reduced renal mass hypertension was due to altered activity of the Na^{+}-K^{+} pump in adrenergic nerve terminals (see background) or to altered activity of the vascular smooth muscle cell Na^{+}-K^{+} pump. Therefore, in another series of experiments,

ouabain-sensitive and insenstive ^{86}Rb uptakes by tail arteries from control normotensive and experimental hypertensive rats were measured following acute in vitro denervation. The method of Aprigliano and Hermsmeyer (1976) was used to produce in vitro denervation.

Tail arteries were obtained from paired experimental hypertensive and control normotensive rats as previously described and then incubated for 10 minutes in 30 ml of a Krebs-Henseleit solution (adjusted to pH 4.9 with glutathione to slow oxidation of the 6-hydroxydopamine) containing 300 ug/ml of 6-hydroxydopamine plus 10^{-6}M phentolamine to block the constrictor effect of catecholamines released in response to the 6-hydroxydopamine (Aprigliano and Hermsmeyer, 1976). Ouabain-sensitive and insensitive ^{86}Rb uptakes were then measured as previously described. Since oxidation products of 6-hydroxydopamine show high absorbance readings at 490 mu (Heikkila and Cohen, 1973), the rate of oxidation of 6-hydroxydopamine was estimated by measuring the change in absorbance (Beckman Model 25 spectrophotometer) at a wavelength of 490 mu between the time the 6-hydroxydopamine was put into solution and the end of the 10 minute incubation period. A change in absorbance of 0.080 units or less is indicative of minimal oxidation of 6-hydroxydopamine (Aprigliano and Hermsmeyer, 1976).

ROLE OF THE ANTEROVENTRAL THIRD VENTRICLE (AV3V) REGION IN THE DEVELOPMENT OF REDUCED RENAL MASS HYPERTENSION, VASCULAR Na^+-K^+ PUMP ACTIVITY, AND CIRCULATING OUABAIN-LIKE FACTOR

Electrolytic lesions of the AV3V region of the brain have been shown to prevent the development of one-kidney, figure eight and one-kidney, DOCA, salt hypertension in rats (Brody et al. 1978). Furthermore, it has been shown that AV3V lesions prevent the appearance of a

circulating sodium transport inhibiting factor following volume expansion (Bealer et al., 1979). Therefore, in another series of experiments the role of the AV3V region of the brain in the mechanism of the development of reduced renal mass hypertension, vascular Na^+-K^+ pump activity, and the appearance of ouabain-like factor in blood was studied.

Lesions of the anterior wall of the third cerebral ventricle (AV3V) were produced in male normotensive Wistar Rats (170-190 g) under sodium pentobarbital (50 mg/kg ip) anesthesia by Dr. James Buggy, University of South Carolina, using his standard technique (Buggy et al., 1977; Brody et al., 1978). Briefly, electrolytic lesions were produced by passing 2 ma direct current for 20 seconds; the lesioning electrode, a 24 gauge nichrome wire insulated, except for 0.5 mm at the beveled tip, was the anode, while an uninsulated steel rod inserted into the rectum served as the cathode. With the skull leveled between bregma and lambda reference points, the lesioning electrode was positioned with stereotaxic guidance on the midline, 0.2 mm caudal to bregma, and 7.2 mm below dura. As a control, other rats underwent a sham lesioning procedure in which the electrode was lowered 5 mm below dura but no current was passed.

Postoperatively, water intake and body weight were monitored daily. Rats with accurately placed AV3V lesions have disrupted regulation of body fluids characterized by an acute dehydration resulting from hypodipsia and lack of compensatory antidiuresis (Brody et al., 1978). Therefore, only those lesioned animals exhibiting one or more days of hypodipsia and a postoperative drop in body weight were selected for study. In these lesioned animals, hydration was maintained during the hypodipsic period by providing access to a palatable fluid such as su-

crose solution or a chocolate solution which was avidly consumed. By three weeks after lesioning, rats had recovered adequate voluntary water intake. Only those animals showing complete hydration recovery (daily water intake similar to pre-lesion level) were used in this study. AV3V lesioned and sham lesioned rats then underwent subtotal nephrectomy as previously described. All rats then consumed a low sodium diet (0.02 percent sodium, 0.59 percent potassium; Bioservices Inc.), drank a one percent NaCl solution and were divided into pairs, each pair consisting of a sham and a lesioned rat.

Systolic blood pressure (tail plethysmography, Natume KN-209) and body weight were then monitored for five weeks. At the end of this five week-period paired sham and lesioned rats were anesthetized with sodium pentobarbital (50 mg/Kg ip), their tail arteries excised for measurement of vascular Na^+-K^+ pump activity (as previously described), six ml of aortic blood collected into a chilled test tube containing 0.2 ml sodium heparin (1000 units/ml) for measurement of ouabain-like activity, and brains from the lesioned animals removed for histological confirmation of lesion placement. Measurement of ouabain-like actvity is described later. For histological examination the brains were blocked and placed in formalin solution for fixation.

Frozen sections (40 micron) were taken through the extent of the lesion, stained with a Nissl stain and examined under light microscope to histologically verify the location of the lesion. The anatomical structures consistently destroyed by AV3V lesion included preoptic periventricular nuclei, the median preoptic nucleus, and the anterior wall of the third ventricle with the associated organum vasculosum of the lamina terminalis; only animals with complete, bilateral damage to

these structures were accepted for statistical evaluation.

ASSAY FOR OUABAIN-LIKE FACTOR IN BLOOD

In another series of experiments, to determine whether a circulating Na^+-K^+ pump inhibiting factor is present in the blood of reduced renal mass rats, tail arteries from untouched normotensive male Wistar rats (350-400 g) were incubated in supernates prepared from the boiled plasma of experimental hypertensive and paired control normotensive reduced renal mass rats and ^{86}Rb uptake measured.

Preparation of Supernates from Boiled Plasma

The preparation of supernates for assay of ouabain-like factor in blood for this study and for previous studies was as follows.

Six ml of aortic blood was collected from each rat into a cooled syringe placed in a jacket packed with ice. The blood was then transferred to to a chilled tube containing 0.2 ml of sodium heparin (1000 units/ml), centrifuged at 1,100 X g for 10 minutes in a refrigerated centrifuge (Sorvall RC-5; SM-24 rotor), and the plasma separated. The plasma was then kept at room temperature for 30 minutes to enhance generation of Na^+-K^+ pump inhibiting factor, if present (Gruber et al., 1978). The plasma was then boiled for five minutes in glass screw-capped tubes to inactivate proteolytic enzymes and to precipitate denatured proteins, centrifuged at 35,596 X g for 90 minutes in a refrigerated centrifuge (Sorvall RC-5; SM-24 Rotor), the supernates collected and frozen (-20°C) until assayed for the presence of a ouabain-like factor. In some rats supernates were also used for composition analysis.

Incubation of Normal Tail Arteries in Supernates

The tail artery from one untouched, normotensive, male Wistar rat was removed under sodium pentobartal anesthesia (75 mg/kg ip), cleaned of

adventitia, opened longitudinally, and cut into one cm long pieces. One half of the tail artery was incubated in supernate prepared from an experimental rat and the other half was incubated in supernate prepared from a paired control rat. Studies with volume expanded animals have shown that a humoral sodium transport inhibiting factor is present after two hours of a maintained volume expansion and that the tail arteries of these rats show suppressed vascular Na^+-K^+ pump activity (Pamnani et al., 1978). Therefore in this study tail arteries were incubated in the respective supernates for two hours at room temperature and ^{86}Rb uptake then measured. Rubidium 86 uptake was measured using our standard technique with some modifications. The detailed methods were as follows.

After the two hour incubation period the test tubes containing the supernates and the incubated tail arteries were transferred to a 37°C shaking water bath (GCA/Precision Scientific) and the standard concentration of ^{86}Rb added. The incubation was then continued for 18 minutes, after which the tissues were removed, immediately washed three times in a K^+-free Krebs-Henseleit solution containing 2 mM "cold" RbCl, blotted, and placed in a gamma scintillation counter (Searle 1185) to determine ^{86}Rb uptake. The tissues were then dried in an oven overnight at 100°C and ^{86}Rb uptake expressed as pMoles/mg tissue dry weight. Due to the small quantity (1.5 to 2.0 ml) of supernate obtained from each animal, only total or ouabain-insensitive ^{86}Rb uptake was measured in each assay.

COMPOSITION ANALYSIS OF SERUM AND SUPERNATES FROM REDUCED RENAL MASS RATS

Six ml of aortic blood was collected from control normotensive and experimental hypertensive reduced renal mass rats, allowed to clot

in polypropylene tubes at room temperature, spun for 10 minutes at 1,100 X g in a refrigerated centrifuge (Sorvall RC-5; SM-24 rotor) and the serum separated for composition analysis.

Supernates of boiled plasma from control normotensive and experimental hypertensive rats were also prepared (as previously descibed) for composition analysis.

Na^+ and K^+ concentrations were measured by flame photometry (Beckman Klina Flame), chloride by chloride titration (Radiometer, Copenhagen, CMT 10 Chloride Titration), osmolality by freezing point depression osmometer (Advanced Instruments, Inc., Model 3D II), creatnine by the spectrophotometric (Beckman Spectrophotometer Model 26) method of Bonsnes and Tausky (1945), blood urea nitrogen by colorimetry (Davidson and Wells, 1962), protein by the biuret method and Ca^{2+} and Mg^{2+} by atomic absorption spectrophotometry (Perkin Elmer 603).

DOCUMENTATION THAT THIS MODEL IS A LOW RENIN, VOLUME-EXPANDED FORM OF HYPERTENSION

To document that the reduced renal mass model of hypertension is a low renin, volume-expanded form of hypertension, plasma renin activity, plasma volumes, and extracellular fluid volumes were measured in control normotensive and experimental hypertensive reduced renal mass rats.

Measurement of Plasma Renin Activity

The method of Haber et al. (1969) was used to measure plasma renin activity. This method measures the ability of plasma renin to convert plasma angiotensinogen to angiotensin I in the presence of converting enzyme inhibitors which prevent the angiotensin I from being converted to angiotensin II. A Becton-Dickinson radioimmunoassay kit was

used. The principle of this radioimmunoassay is as follows: Unlabeled angiotensin I competes with labeled angiotensin I for a limited number of available antibody binding sites. The level of radioactive label bound is therefore inversely proportional to the concentration of angiotensin I in the plasma sample or standard. The angiotensin I tracer is labeled with ^{125}I on the tyrosine residue.

After four weeks of sustained hypertension in the experimental (saline drinking) reduced renal mass rats, and a similar time period in the controls (distilled water drinking), the animals were decapitated and three ml of blood collected in cold polypropylene tubes, containing five mg of EDTA. The blood was then centrifuged at 1,100 x g for 10 minutes in a refrigerated centrifuge (Sorvall RC-5; SM-24 Rotor), the supernatant pipetted into pre-chilled tubes in the cold room, and the sample rapidly frozen in dry ice.

Inhibitors of converting enzyme and angiotensinases were added to the plasma samples (buffered with tris acetate, pH 7.4). Angiotensin I was then generated for three hours at 37°C. A constant amount of ^{125}I tracer and antiserum was then added to each sample and to known amounts of angiotensin I standard. The samples were then incubated for 24 hours at $^{+}2°C$. Bound and free angiotensin was then separated by contact with dextran-coated charcoal which absorbs the free antigen molecules. Centrifugation (RC-3B; SM-6000 swinging bucket Rotor), in the cold, pellets the charcoal leaving antibody bound angiotensin I in the supernatant. Each sample was then counted in a gamma scintillation counter (Searle 1185) and compared to a standard curve for determination of angiotensin I in the plasma sample. Plasma renin activity was expressed as ng/ml/hr of angiotensin I generated. The standard curve was

prepared as follows.

Eight tubes, each containing one of the following concentrations of angiotensin I (ng/tube) were prepared: 0.000, 0.020, 0.040, 0.080, 0.125, 0.250, 0.500. These tubes were then treated the same as described for the plasma samples, and radioactive counts determined in a scintillation counter (Searle 1185). A standard curve was then generated by plotting counts vs. ng Angiotensin I per tube.

Measurement of Plasma and Extracellular Fluid Volumes

In another series of experiments, plasma volume and extracellular fluid volumes were measured after four weeks of sustained hypertension in the experimental (saline drinking) reduced renal mass rats and after a similar time period in the control (distilled water drinking) reduced renal mass rats. Evans blue (Fisher Products Inc.) and sodium thiocyanate (Fisher Products Inc.) were used to determine plasma and extracellular fluid volumes, respectively, in the same animals. The dye dilution method of Wang (1959) was used for plasma volume determinations and the method of Elkinton and Taffel (1942) was used for measurement of extracellular fluid volume. Evans blue binds plasma proteins and does not enter cells or readily leave the capillaries whereas sodium thiocyanate passes readily through capillaries. Evans blue distributes evenly in 10 minutes while sodium thiocyanate requires one hour of mixing *in vivo* to become evenly distributed. Both of these substances can be measured spectrophotometrically, Evans blue at a wavelength of 605 mu and sodium thiocyanate at a wavelength of 490 mu. Therefore these two substances can be used together, as one does not interfere with the absorbance reading of the other.

Rats were fasted for 12 hours (but had free access to their

respective drinking fluids) before volume measurements. They were then anesthetized with sodium pentobarbital (75 mg/kg ip) and the trachea cannulated (PE 240, Clay Adams) to facilitate respiration. The external jugular vein (PE 50, Clay Adams) carotid artery (PE 60, Clay Adams) and urinary bladder (PE 50, Clay Adams) were then cannulated and a urine sample collected for determining background absorbance values. Sodium heparin (100 units in 0.1 ml, Porcine Mucosal, Lypho-Med, Inc.) was injected intravenously for systemic anticoagulation and the animal allowed to stabalize for 20 minutes. A 0.6 ml blood sample was then taken through the carotid artery cannula with a 1 ml syringe, spun for two minutes in a microcentrifuge (Beckman Microfuge B), and 0.3 ml of plasma supernate removed for determining background absorbance values. The cells were then resuspended in 0.3 ml of normal saline and reinfused into the rat via the jugular vein cannula. This cannula was then flushed with 0.1 ml of normal saline and the animals allowed to stabilize for 20 minutes.

The urinary bladder was then emptied, 0.5 ml of one percent sodium thiocyanate solution (made up in normal saline) injected via the jugular vein cannula and the cannula flushed with 0.1 ml of normal saline. Fifty minutes later 0.3 ml of 0.45 percent Evans blue was injected via the jugular vein cannula and the cannula flushed with 0.1 ml of normal saline. Ten minutes after the injection of Evans blue, 1.0 ml of blood was collected through the carotid artery cannula and spun in a microcentrifuge for two minutes to separate the plasma for determination of Evans blue and sodium thiocyanate concentrations. During this time urine volume was determined and a urine sample collected.

For Evans blue determination, 0.1 ml of plasma was added to 0.9

ml of saline and absorbance at a wavelength of 605 mu determined in a spectrophotometer (Beckman Model 25). This value was then compared to a standard curve (preparation described later) and plasma concentration of Evans blue calculated. Urinary concentration of Evans blue was determined in the same manner.

Plasma sodium thiocyanate concentration was determined as follows: 0.2 ml of plasma was added to 0.6 ml of 10 percent trichloroacetic acid (TCA) to precipitate proteins, spun in a microcentrifuge (Beckman Microfuge B) for two minutes and the supernate collected. A 0.4 ml sample of this supernate was added to 0.4 ml of distilled water containing 0.2 ml of ferric nitrate reagent (25g $Fe(NO_3)_3 \cdot 9H_2O$ + 12.5 ml HNO_3 + 500 ml distilled H_2O). Absorbance was then determined spectrophotometrically (Beckman Model 25) at a wavelength of 490 mu and compared to a standard curve (preparation described later) for calculation of plasma concentration. Urinary sodium thiocyanate concentration was determined by adding 0.25 ml of urine to 3.75 ml of distilled water containing 1.0 ml of ferric nitrate reagent and the absorbance at a wavelength of 490 mu determined.

Spectrophotometer blanks for Evans blue plasma and urine determinations were saline. The sodium thiocyanate plasma blank was made by combining 0.5 ml of distilled water with 0.3 ml of 10 percent TCA plus 0.2 ml of ferric nitrate reagent. The urine blank for thiocyanate consisted of 4.0 ml of distilled water plus 1.0 ml of ferric nitrate reagent.

Evans blue and sodium thiocyanate standard curves were generated by measuring the absorbance of known quantities of Evans blue or sodium thiocyanate, respectively, and then plotting absorbance vs concentration.

A known amount of Evans blue or sodium thiocyanate was added to a test tube and these samples were then treated the same as their respective plasma samples. The concentrations of Evans blue used were 11.25 mg/ml, 9 mg/ml, 7.5 mg/ml, 5.625 mg/ml, 4.5 mg/ml, 3.0 mg/ml and, 2.25 mg/ml. The concentrations of sodium thiocyanate used were 1.000 g/ml, 0.200 g/ml, 0.133 g/ml, 0.100 g/ml, 0.050 g/ml, 0.025 g/ml, 0.020 g/ml, 0.0167 g/ml, 0.0125 g/ml, and 0.100 g/ml.

The following equation was used for calculation of absolute plasma and extracellular fluid volumes:

$$\text{volume} = \frac{\text{amount injected (mg)} - \text{amount excreted (mg)}}{\text{plasma concentration (mg/ml)}}$$

Relative plasma and extracellular fluid volumes were then expressed as ml/Kg body weight.

In another group of control and experimental reduced renal mass rats extracellular fluid volumes were measured with ^{14}C inulin (New England Nuclear)

For this series of experiments the rats were anesthetized with sodium pentobarbital (75 mg/Kg ip) and surgically prepared as in the experiments using sodium thiocyanate. The animals were allowed to recover for 20 minutes after surgery. Each rat was then given an intravenous injection of 2.5 uCi of ^{14}C inulin/100 g body weight via the jugular vein cannula and the cannula flushed with 0.1 ml of normal saline. One hour later a 1.0 ml blood sample was collected and the plasma separated by centrifugation. Samples of 100 ul and of 200 ul of plasma were then added to 10 ml of Aquasol and counted for 10 minutes or 10^6 counts in a Tri-Carb liquid scintillation counter (Searle Analytic 81). Radioactivity of the infusate was similarly measured.

The following equation was used for calculating absolute extracellular fluid volumes.

$$\text{volume} = \frac{\text{DPM injected} - \text{DPM excreted}}{\text{plasma concentration (DPM/ml)}}$$

Relative extracellular fluid volumes were expressed ml/kg body weight.

STATISTICAL METHODS

Statistical analysis with paired student's t test was used to compare ^{86}Rb uptakes and microsomal ATPase activities in tail arteries and hearts, respectively, obtained from experimental and paired control animals. Values of $p < .05$ were considered statistically significant.

The student's t test was used to statistically analyze arterial blood pressures, body weights, heart weight/body weight ratios, plasma renin activity, hematocrits, body fluid volumes, levels of plasma and supernate constituents, plasma and brain catecholamine levels, metabolic data on sodium and fluid intake, and apparent k_m values for RbCl, obtained from experimental and control group animals.

Apparent k_m values for RbCl were estimated from a plot of $(RbCl)^2$/uptake vs. $(RbCl)^2$ (Dowd and Riggs, 1965).

RESULTS

REDUCED RENAL MASS RATS - GENERAL DATA

General data on the subtotally nephrectomized rats used in this study are presented in Table 1. Body weights and systolic blood pressures of the control and experimental rats were not significantly different before subtotal nephrectomy. Substitution of saline for drinking in the experimental rats following subtotal nephrectomy significantly increased their daily sodium intake compared to control (distilled water drinking) rats (Table 2). Five weeks after subtotal nephrectomy and substitution of saline for drinking, mean systolic blood pressure and heart weight/body weight ratio in the experimental rats were significantly higher and their body weights significantly lower than in the distilled water drinking control rats. Also at this time, hematocrits of the experimental rats were significantly lower than control rats.

Figures 1 and 2 show the progressive changes in body weights and systolic blood pressures, respectively of these two groups of animals during the five weeks following subtotal nephrectomy. Body weight increased in all rats over the time course of the study, but the weekly increase was greater in the control rats than in the experimental rats, with the result that at two through five weeks following subtotal nephrectomy the control rats weighed significantly more than the experimental rats. Systolic blood pressure of the control rats did not change during the study and remained at about 116 mmHg. Systolic blood pressure of the experimental rats increased progressively following subtotal nephrectomy and was significantly greater than the systolic blood pres-

TABLE 1. Group means ± SE of body weights and systolic blood pressures (tail plethysmography) of control and experimental rats before and five weeks after subtotal nephrectomy; also, hematocrits and heart weight/body weight ratios of control and experimantal rats five weeks after subtotal nephrectomy.

Table 1

GENERAL DATA FROM REDUCED RENAL MASS RATS

	CONTROL RATS	EXPERIMENTAL RATS
BEFORE SUBTOTAL NEPHRECTOMY		
BODY WEIGHT (g)		
N = 50	210.5 ± 3.6	217.2 ± 4.1
SYSTOLIC BLOOD PRESSURE (mmHg)		
N = 50	116.4 ± .9	115.5 ± .9
FIVE WEEKS AFTER SUBTOTAL NEPHRECTOMY		
BODY WEIGHT (g)		
N = 50	299.1 ± 5.2	271.5 ± 4.4[a]
SYSTOLIC BLOOD PRESSURE (mmHg)		
N = 50	118.6 ± .9	168.0 ± 1.0[a]
HEMATOCRITS (%)		
N = 20	41.9 ± .7	32.7 ± 1.1[a]
HEART WEIGHT/BODY WEIGHT RATIO (mg/g)		
N = 8	2.30 ± .10	3.30 ± .12[a]

a $p \leq .005$ EXPERIMENTAL COMPARED TO CONTROL

TABLE 2. Group means ± SE of average daily sodium intake of experimental rats after subtotal neprectomy. Average daily sodium intake of control rats was always less than 1 mEq following subtotal nephrectomy.

Table 2

AVERAGE DAILY SODIUM INTAKE OF REDUCED RENAL MASS RATS

DAYS AFTER SUBTOTAL NEPHRECTOMY	SODIUM INTAKE (mEq/DAY) CONTROL RATS N = 10	EXPERIMENTAL RATS N = 10
1	<1	4.8 ± .2
2	<1	7.0 ± .2
3	<1	8.4 ± .4
7	<1	11.6 ± .4
14	<1	18.1 ± .5
21	<1	17.6 ± .3
28	<1	17.3 ± .5
35	<1	17.2 ± .4

FIGURE 1. Average weekly body weights of control and experimental rats before and for five weeks after subtotal nephrectomy.

BODY WEIGHTS OF REDUCED RENAL MASS RATS

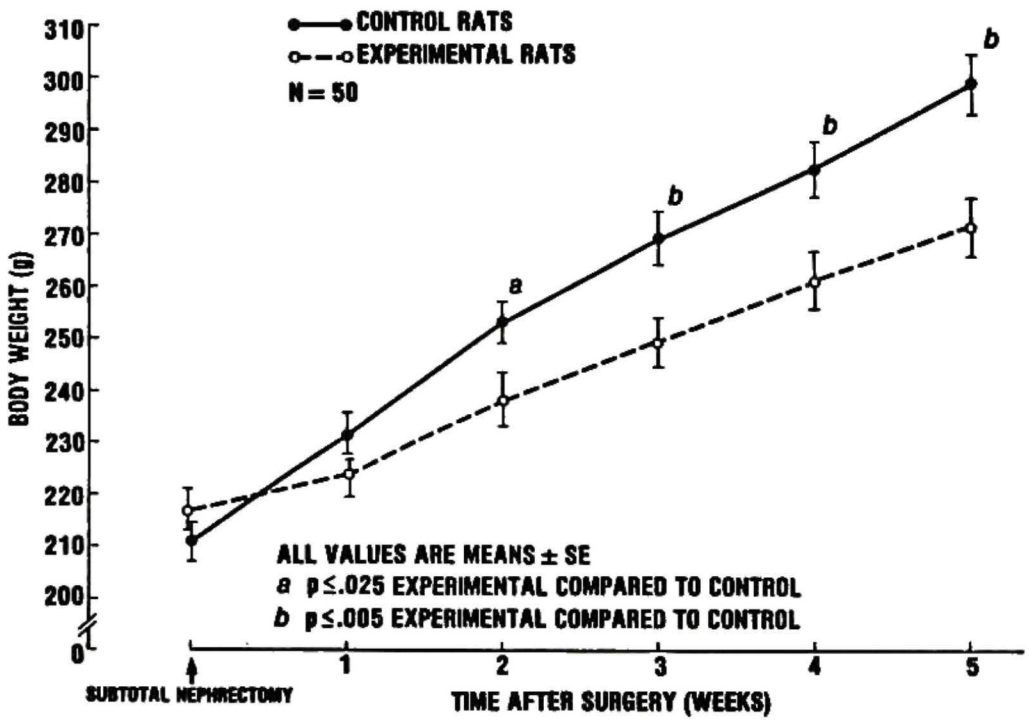

Figure 1

FIGURE 2. Average weekly systolic blood pressures (tail plethysmography) of control and experimental rats before and for five weeks after subtotal nephrectomy.

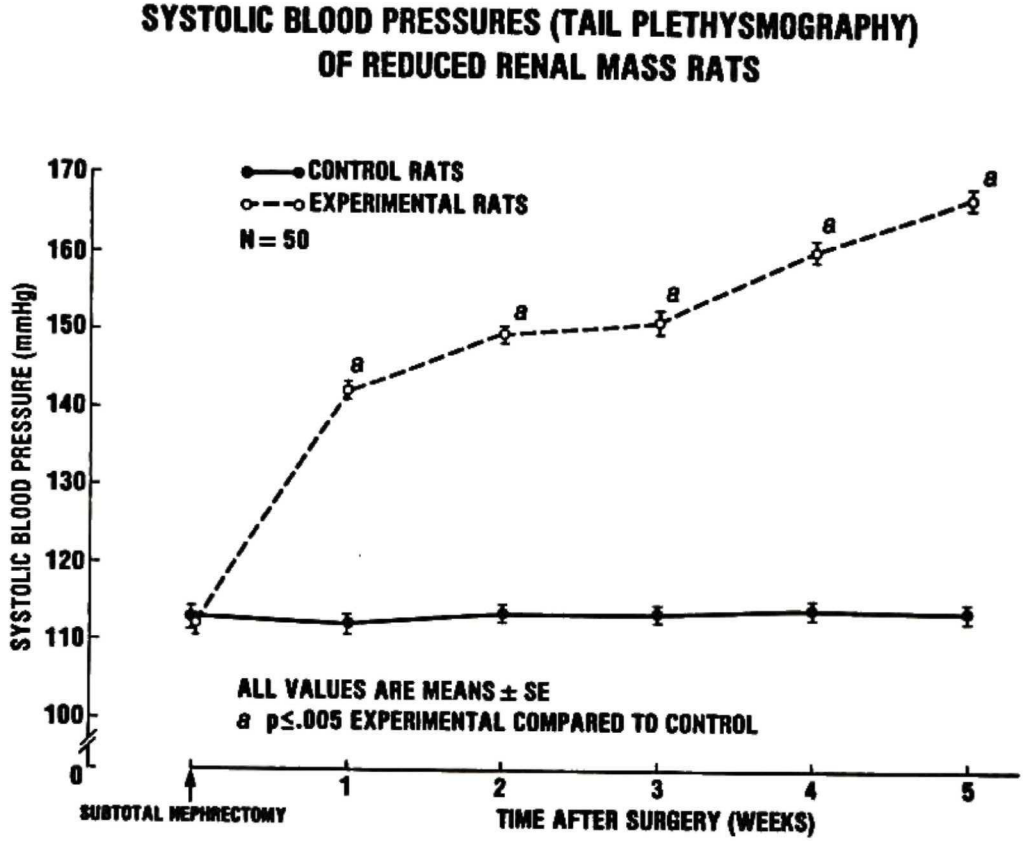
SYSTOLIC BLOOD PRESSURES (TAIL PLETHYSMOGRAPHY)
OF REDUCED RENAL MASS RATS
CONTROL RATS
EXPERIMENTAL RATS
N = 50
SYSTOLIC BLOOD PRESSURE (mmHg)
170
160
150
140
130
120
110
100
0
a
ALL VALUES ARE MEANS ± SE
a p≤.005 EXPERIMENTAL COMPARED TO CONTROL
SUBTOTAL NEPHRECTOMY
1
2
3
4
5
TIME AFTER SURGERY (WEEKS)

Figure 2

sure of the control rats from the first through the fifth week after subtotal nephrectomy. By the fifth week it had reached 168 ± 1 mmHg.

Presented in Table 3 are the systolic, diastolic, and mean arterial blood pressures of control and experimental rats as measured in the conscious state via carotid artery cannulas. These measurements were obtained just before the animals were terminated for assay. Compared to control rats, systolic, diastolic, and mean arterial blood pressures of the experimental rats were significantly higher, thus confirming our systolic blood pressure results obtained by the indirect plethysmographic method.

CARDIOVASCUALR MUSCLE CELL Na^+-K^+ PUMP ACTIVITY

Rubidium Uptake by Blood Vessels

Ouabain-sensitive and insensitive ^{86}Rb uptakes by tail arteries from subtotally nephrectomized control normotensive and paired experimental hypertensive rats are presented in Figure 3. Ouabain-sensitive ^{86}Rb uptake was decreased by 40 percent ($p < .005$, N=10) in tail arteries from the experimental rats compared to uptake by tail arteries from control rats. Ouabain-insensitive ^{86}Rb uptakes however, were not significantly different in the two groups of animals.

Data from ouabain inhibition experiments is presented in figure 4. The percent inhibition of ouabain-sensitive ^{86}Rb uptake at all concentrations of ouabain was the same in tail arteries from control and experimental rats, indicating that the observed pump suppression in the experimental rats was not due differences in sensitivity of the vessels to ouabain.

The apparent k_m values obtained from the Rb uptake kinetic studies were 0.42 ± .01 and 0.43 ± .01 in tail arteries from control and

TABLE 3. Group means ± SE of direct (carotid artery cannula) systolic, diastolic, and mean arterial blood pressures plus indirect (tail plethysmography) systolic blood pressures of control and experimental rats.

Table 3

DIRECT vs INDIRECT BLOOD PRESSURES OF REDUCED RENAL MASS RATS

	CONTROL RATS	EXPERIMENTAL RATS
DIRECT BLOOD PRESSURES (mmHg)		
SYSTOLIC	121.0 ± 2.7	174.5 ± 2.5
DIASTOLIC	105.0 ± 2.7	140.0 ± 1.6
MEAN	111.5 ± 2.4	159.0 ± 1.3
INDIRECT BLOOD PRESSURES (mmHg)		
SYSTOLIC	118.5 ± 1.3	168.0 ± 1.8
	N = 3	N = 3

FIGURE 3. Ouabain-sensitive and insensitive ^{86}Rb uptakes by tail arteries from control normotensive and experimental hypertensive reduced renal mass rats.

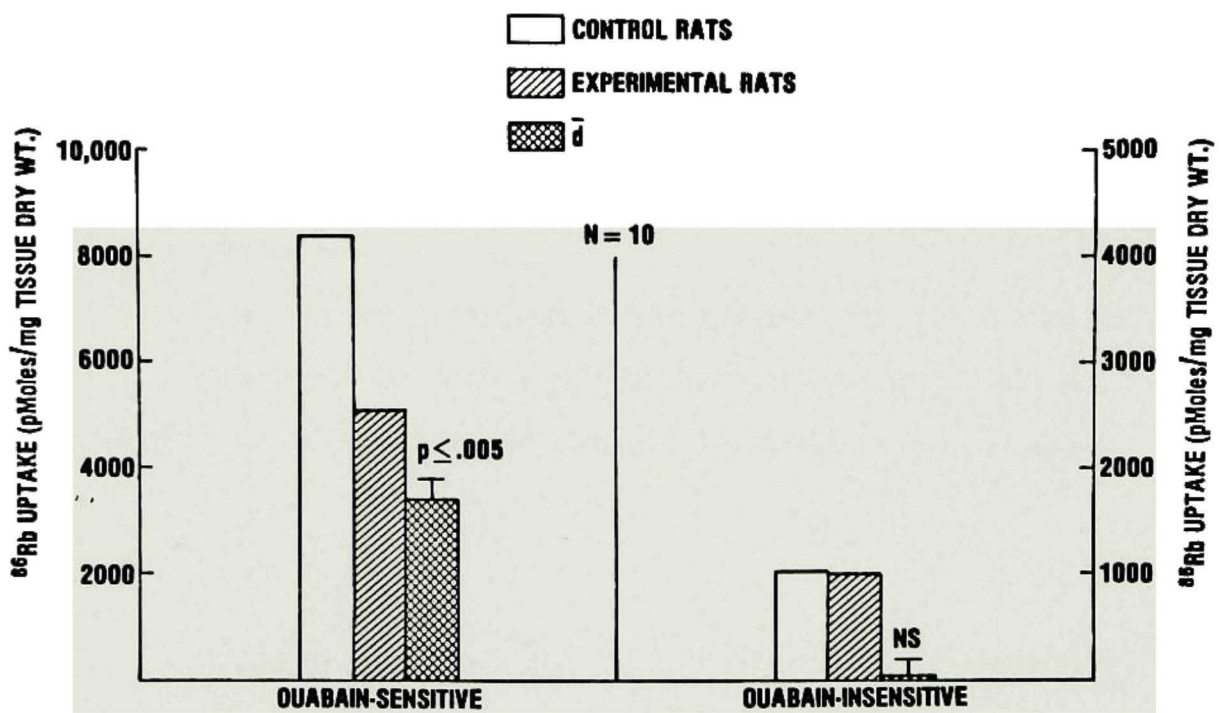
^{86}Rb UPTAKE BY TAIL ARTERIES FROM REDUCED RENAL MASS RATS
CONTROL RATS
EXPERIMENTAL RATS
^{86}Rb UPTAKE (pMoles/mg TISSUE DRY WT.)
10,000
8000
6000
4000
2000
5000
4000
3000
2000
1000
N = 10
p≤.005
NS
OUABAIN-SENSITIVE
OUABAIN-INSENSITIVE

Figure 3

FIGURE 4. Percent of ouabain-sensitive ^{86}Rb uptake by tail arteries from control normotensive and experimental hypertensive reduced renal mass rats in the presence of varying amounts of ouabain.

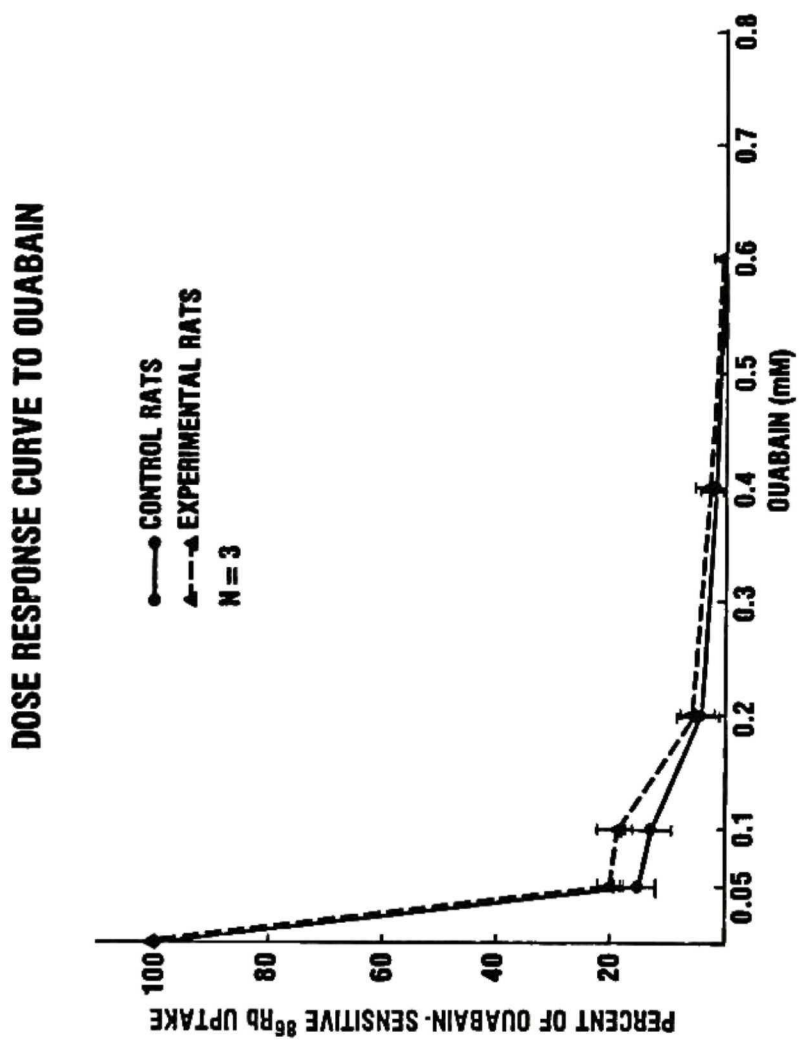
DOSE RESPONSE CURVE TO OUABAIN
CONTROL RATS
EXPERIMENTAL RATS
N = 3
PERCENT OF OUABAIN-SENSITIVE 86Rb UPTAKE
100
80
60
40
20
0.05
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
OUABAIN (mM)

Figure 4

experimental rats, respectively, thus indicating that the observed pump suppression in the experimental rats was not due to altered rubidium affinity.

Microsomal Na^+, K^+-ATPase Activity in Myocardium

There were also significant difference in myocardial Na^+, K^+-ATPase activity in these two groups of animals (Figure 5). Na^+, K^+-ATPase activity of microsomes prepared from experimental hypertensive rats was decreased by 18 percent ($p < .025$, N=10) compared to microsomes prepared from myocardium of control normotensive rats. However, Mg^{2+}-ATPase activity was significantly higher in cardiac microsomes prepared from the experimental rats compared to cardiac microsomes prepared from control rats ($p < .005$, N=10)

Figure 6 presents the sialic acid contents of microsomes prepared from myocardium of control normotensive and experimental hypertensive reduced renal mass rats. Sialic acid content of microsomes from these two groups of rats were not significantly different.

TIME STUDY

Figure 7 compares the progressive increase in blood pressure with changes in vascular Na^+-K^+ pump activity of subtotally nephrectomized (saline drinking) experimental rats. In this graph, ouabain-sensitive ^{86}Rb uptake by tail arteries from experimental hypertensive rats (expressed as percent of the uptake by tail arteries from control normotensive rats) is plotted on the left ordinate and systolic blood pressure of these experimental rats is plotted on the right ordinate against time following subtotal nephrectomy and substitution of saline for drinking on the abscissa. One week after subtotal nephrectomy the experimental rats were hypertensive (systolic blood pressure 143.5 ± 1.0

FIGURE 5. Na^+,K^+-ATPase and Mg^{2+}-ATPase activities in cardiac microsomes prepared from control normotensive and experimental hypertensive reduced renal mass rats.

ATPase ACTIVITY IN CARDIAC MICROSOMES PREPARED FROM REDUCED RENAL MASS RATS

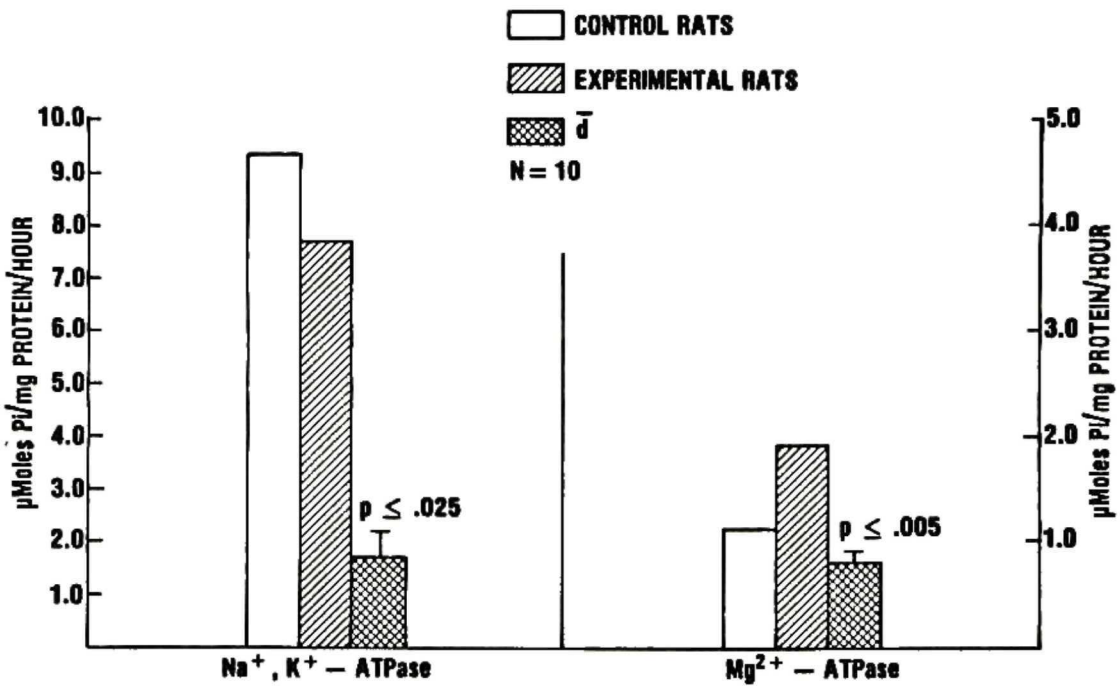

Figure 5

FIGURE 6. Sialic acid content of cardiac microsomes from control normotensive and experimental hypertensive reduced renal mass rats.

SIALIC ACID CONTENT OF CARDIAC MICROSOMES FROM REDUCED RENAL MASS RATS

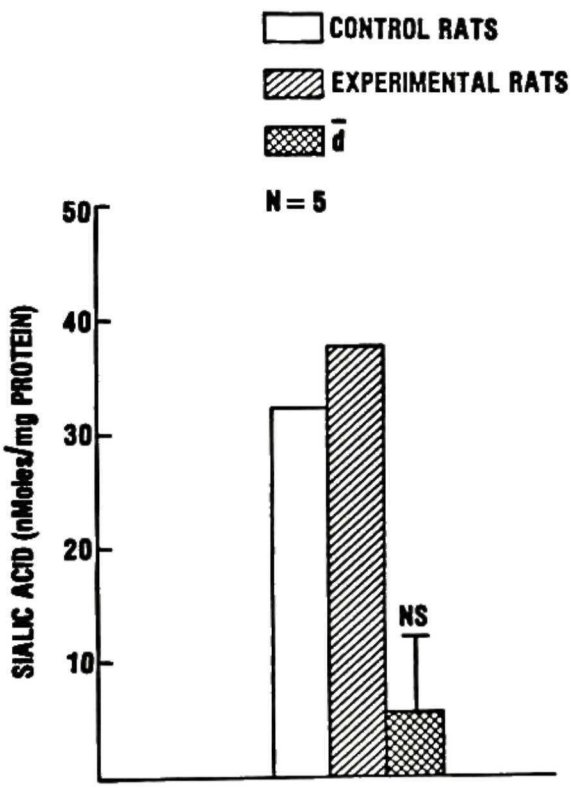

Figure 6

FIGURE 7. Ouabain-sensitive ^{86}Rb uptake by tail arteries from experimental hypertensive reduced renal mass rats expressed as percent uptake by tail arteries from control normotensive reduced renal mass rats (left ordinate) and systolic blood pressure (tail plethysmography) of experimental hypertensive reduced renal mass rats (right ordinate) vs time after subtotal nephrectomy (abcissa).

VASCULAR OUABAIN-SENSITIVE ^{86}Rb UPTAKE AND SYSTOLIC BLOOD PRESSURE (TAIL PLETHYSMOGRAPHY) OF REDUCED RENAL MASS HYPERTENSIVE RATS

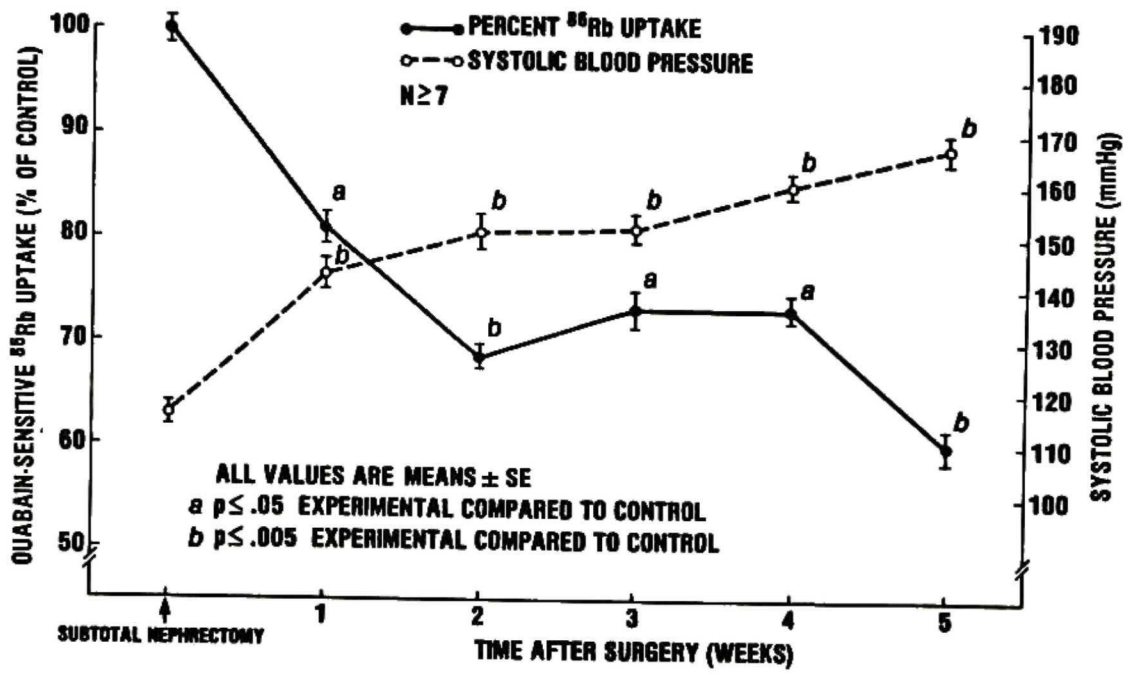

Figure 7

mmHg) and ouabain-sensitive ^{86}Rb uptake was already depressed by 19 percent ($p < .025$, N=7). For the remainder of this study ouabain-sensitive ^{86}Rb uptake by tail arteries from experimental rats remained depressed while the systolic blood pressure of these rats remained elevated. Five weeks after subtotal nephrectomy when systolic blood pressure of the experimental rats was 168.0 ± 1.0 mmHg, ouabain-senstive ^{86}Rb uptake by tail arteries from these rats was decreased by 40 percent ($p < .005$, N=10) compared to arteries from their paired control rats.

Figure 8 is a scatter diagram of ouabain-sensitive ^{86}Rb uptake by tail arteries from experimental hypertensive rats (expressed as percent of the uptake by tail arteries from control normotensive rats) on the ordinate against systolic blood pressure of the experimental rats on the abscissa. Each point represents the systolic blood pressure and percent inhibition of vascular Na^+-K^+ pump activity in one of the rats from the time study. As can be seen, there is no simple quantitative relationship between suppression of pump activity and level of arterial blood pressure.

EFFECT OF SUPERNATES ON RUBIDIUM UPTAKE

Figure 9 shows the effect of supernates prepared from the boiled plasma of control normotensive and paired experimental hypertensive rats on ^{86}Rb uptake by tail arteries obtained from normal untouched Wistar rats. Total ^{86}Rb uptake was decreased by 25 percent ($p < .005$, N=10) when the arteries were incubated in supernates obtained from experimental hypertensive rats compared to those incubated in supernates obtained from control normotensive rats. Ouabain-insensitive uptakes however were not different whether arteries were incubated in supernates obtained from control or experimental rats (Figure 10).

FIGURE 8. Scatter diagram. Each point represents the ouabain-sensitive ^{86}Rb rubidium uptake by the tail artery of one experimental hypertensive reduced renal mass rat and the same animals systolic blood pressure. These are the same animals and data as reported in Figure 7.

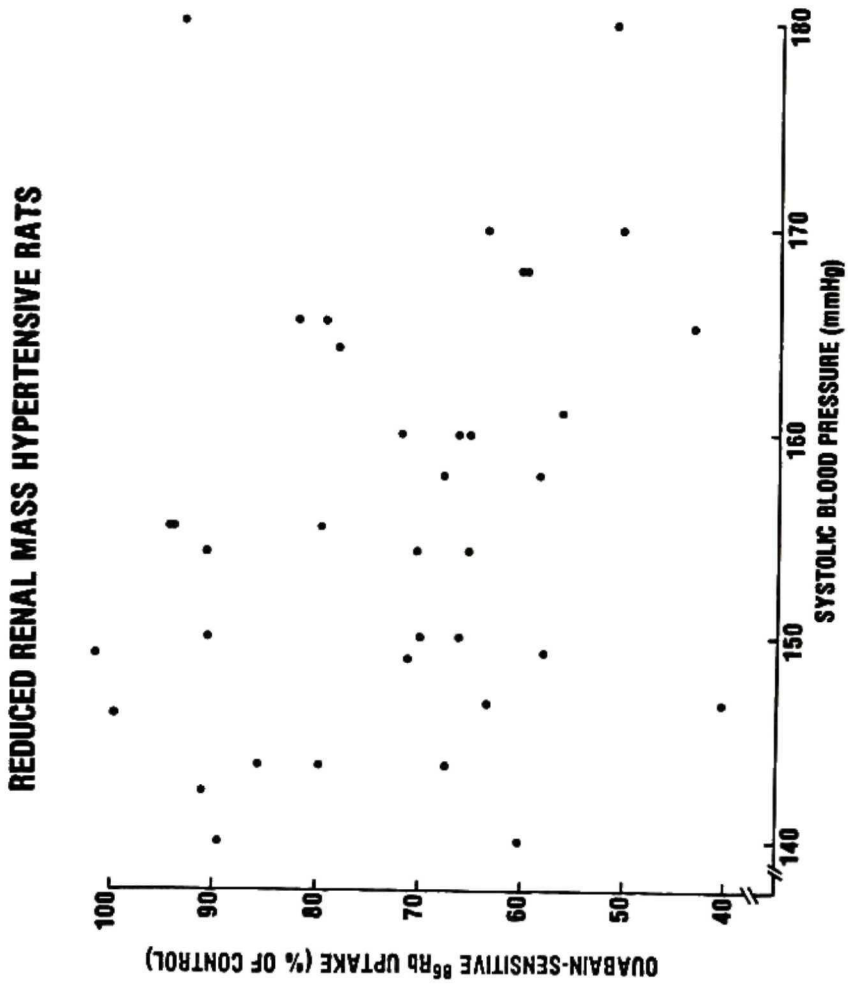
REDUCED RENAL MASS HYPERTENSIVE RATS
OUABAIN-SENSITIVE ^{86}Rb UPTAKE (% OF CONTROL)
100
90
80
70
60
50
40
140
150
160
170
180
SYSTOLIC BLOOD PRESSURE (mmHg)

Figure 8

FIGURE 9. Total ^{86}Rb uptake by tail arteries from normal untouched rats incubated in supernates prepared from boiled plasma of control normotensive and experimental hypertensive reduced renal mass rats.

TOTAL ^{86}Rb UPTAKE BY NORMAL TAIL ARTERIES INCUBATED IN SUPERNATES PREPARED FROM REDUCED RENAL MASS RATS

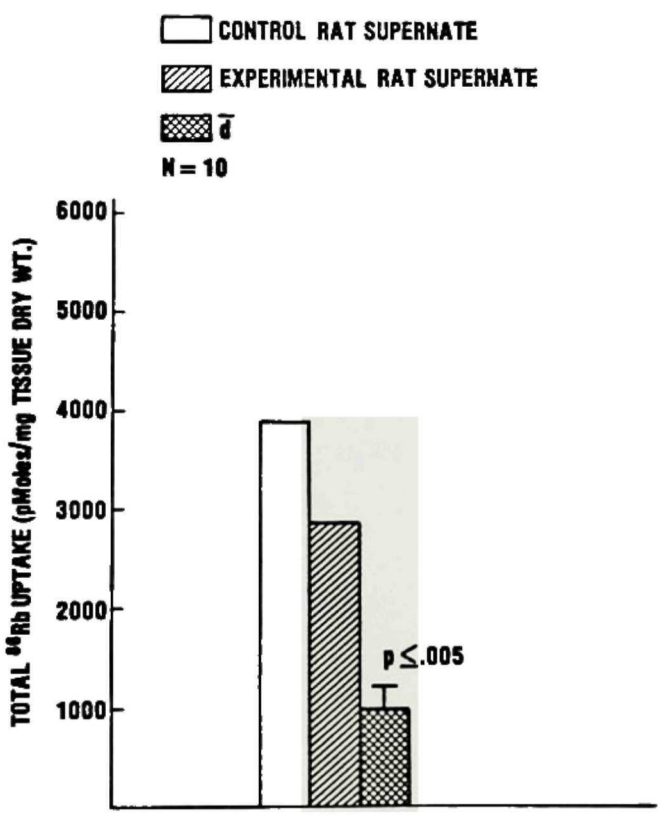

Figure 9

FIGURE 10. Ouabain-insensitive ^{86}Rb uptake by tail arteries from normal untouched rats incubated in supernates prepared from boiled plasma of control normotensive and experimental hypertensive reduced renal mass rats.

OUABAIN-INSENSITIVE ^{86}Rb UPTAKE BY NORMAL TAIL ARTERIES INCUBATED IN REDUCED RENAL MASS RAT SUPERNATES

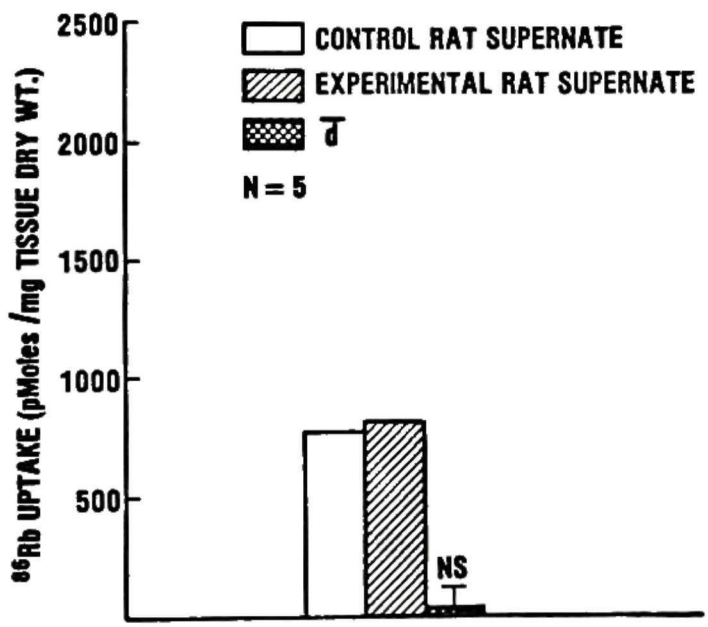

Figure 10

Table 4 presents the composition of supernates obtained from control normotensive and experimental hypertensive rats. Na^+, K^+, Cl^-, osmolality, creatinine, blood urea nitrogen, protein, Ca^{2+}, and Mg^{2+} values were not different in supernates obtained from these two groups of animals.

ROLE OF SYMPATHETIC NERVOUS SYSTEM IN REDUCED RENAL MASS HYPERTENSION

Effect of Peripheral Sympathectomy

Sympathectomy by adrenal demedullation and intravenous administration of 6-hydroxydopamine before subtotal nephrectomy did not alter the development of hypertension or inhibition of Na^+-K^+ pump activity in experimental rats.

Presented in Figures 11 and 12 are the average weekly body weights and systolic blood pressures, respectively of control (distilled water drinking) and experimental (saline drinking) reduced renal mass rats with peripheral sympathectomy. Although the experimental rats tended to weigh less than the control rats following subtotal nephrectomy, body weights of the control and experimental sympathetomized rats did not significantly differ at any time during the study. Body weights of sympathectomized control and experimental rats at five weeks after subtotal nephrectomy were similar to those of control and experimental rats without sympathectomy (compare Figure 1).

Although systolic blood pressures tended to be lower following the last intravenous injection of 6-hydroxydopamine in both groups, these values were not significantly different compared to pre-injection values. As in non-sympathectomized experimental rats, systolic blood pressure of the peripherally sympathectomized experimental rats progressively increased following subtotal nephrectomy and reached an average

Table 4

COMPOSITION OF SUPERNATES FROM CONTROL AND EXPERIMENTAL REDUCED RENAL MASS RATS

	CONTROL RATS	EXPERIMENTAL RATS	
Na^+ (mEq/l)	142.1 ± 1.8	146.1 ± 3.2	N = 7
K^+ (mEq/l)	3.8 ± .2	3.8 ± .4	N = 7
Cl^- (mEq/l)	113.2 ± 2.3	118.9 ± 3.5	N = 7
OSMOLALITY (mOsm)	318.5 ± 5.3	322.7 ± 11.1	N = 7
BUN (mg%)	38.2 ± 5.4	32.4 ± 4.2	N = 6
CREATININE (mg%)	0.89 ± .05	0.88 ± .08	N = 4
PROTEIN (g%)	0.56 ± .04	0.63 ± .04	N = 7
Ca^{2+} (mEq/l)	2.9 ± .8	2.7 ± .4	N = 7
Mg^{2+} (mEq/l)	1.1 ± .2	1.1 ± .1	N = 7

TABLE 4. Group means ± SE of supernate composition from control normotensive and experimental hypertensive reduced renal mass rats.

FIGURE 11. Average weekly body weights of adrenal demedullated control and experimental rats before and after intravenous injection of 6-hydroxydopamine and for five weeks after subtotal nephrectomy.

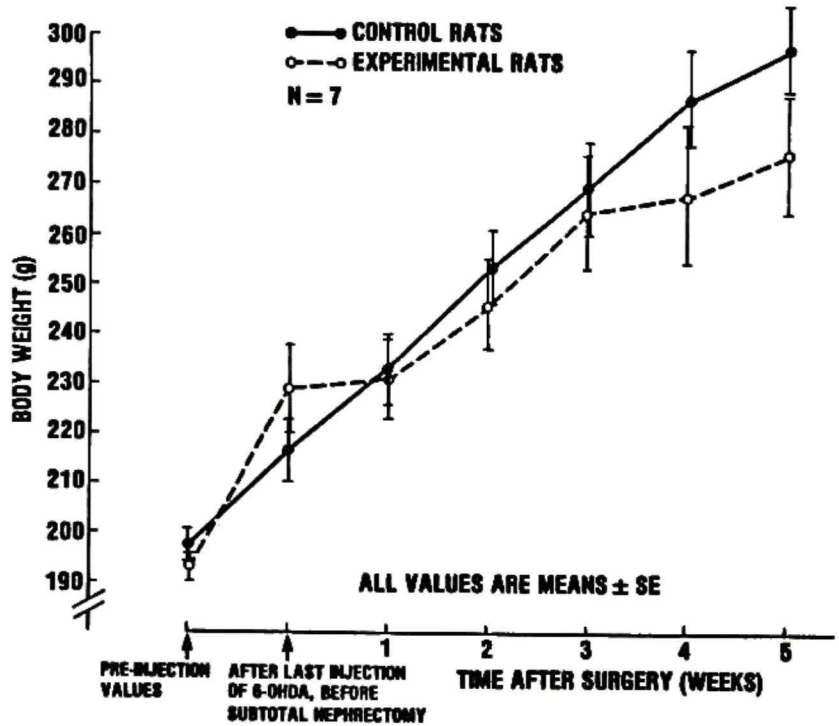
BODY WEIGHTS OF REDUCED RENAL MASS RATS WITH PERIPHERAL SYMPATHECTOMY
CONTROL RATS
EXPERIMENTAL RATS
N = 7
BODY WEIGHT (g)
300
290
280
270
260
250
240
230
220
210
200
190
ALL VALUES ARE MEANS ± SE
PRE-INJECTION VALUES
AFTER LAST INJECTION OF 6-OHDA, BEFORE SUBTOTAL NEPHRECTOMY
1
2
3
4
5
TIME AFTER SURGERY (WEEKS)

Figure 11

FIGURE 12. Average weekly systolic blood pressures (tail plethysmography) of adrenal demedullated control and experimental rats before and after intravenous injection of 6-hydroxydopamine and for five weeks after subtotal nephrectomy.

SYSTOLIC BLOOD PRESSURES (TAIL PLETHYSMOGRAPHY) OF REDUCED RENAL MASS RATS WITH PERIPHERAL SYMPATHECTOMY

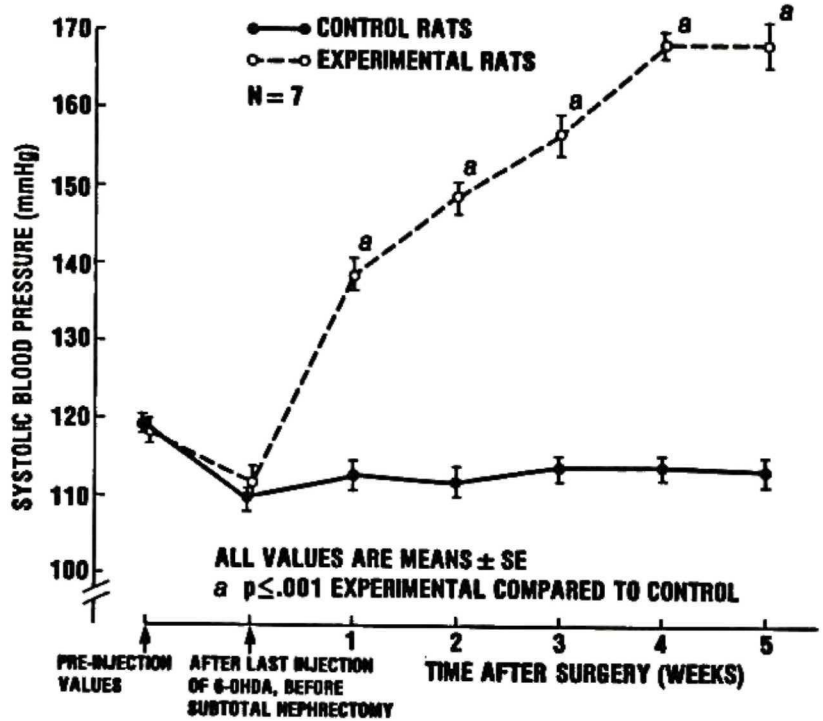

Figure 12

value of 165 ± 1 mmHg by the fifth week after nephrectomy while the control rats remained normotensive. Systolic blood pressure of the sympathectomized experimental rats was significantly greater than that of the sympathectomized control rats from the first through the fifth week following subtotal nephrectomy.

Bar diagrams in Figure 13 represent oubain-sensitive and insensitive ^{86}Rb uptakes by tail arteries obtained from these peripherally sympathectomized, subtotally nephrectomized experimental and control rats. Compared to tail arteries from control rats, ouabain-sensitive ^{86}Rb uptake was decreased by 20 percent ($p < .02$, N=5) in tail arteries from experimental rats. Ouabain-insensitive ^{86}Rb uptakes again were not significantly different.

Presented in Figure 14 are plasma catecholamine levels of the control and experimental peripherally sympathectomized reduced renal mass rats (measured five weeks after subtotal nephrectomy). Since all rats used in this study were adrenal demedullated only norepinephrine was measured by this radioimmunoassay. Plasma norepinephrine levels were not significantly different in the control and experimental rats.

Effect of Central Sympathectomy

In contrast to our findings in peripherally sympathectomized rats, central sympathectomy by intraventricular injection of 6-hydroxydopamine in the brain prevented the development of reduced renal mass hypertension in experimental (saline drinking) rats and abolished depression of vascular Na^+-K^+ pump activity in tail arteries of these rats (Figures 16 and 17). Furthermore, the level of ouabain-like humoral factor was not significantly different in control and experimental, centrally sympathectomized rats (Figure 18).

FIGURE 13. Ouabain-sensitive and ouabain-insensitive ^{86}Rb uptakes by tail arteries from adrenal demedullated, peripherally sympathectomized control normotensive and experimental hypertensive reduced renal mass rats.

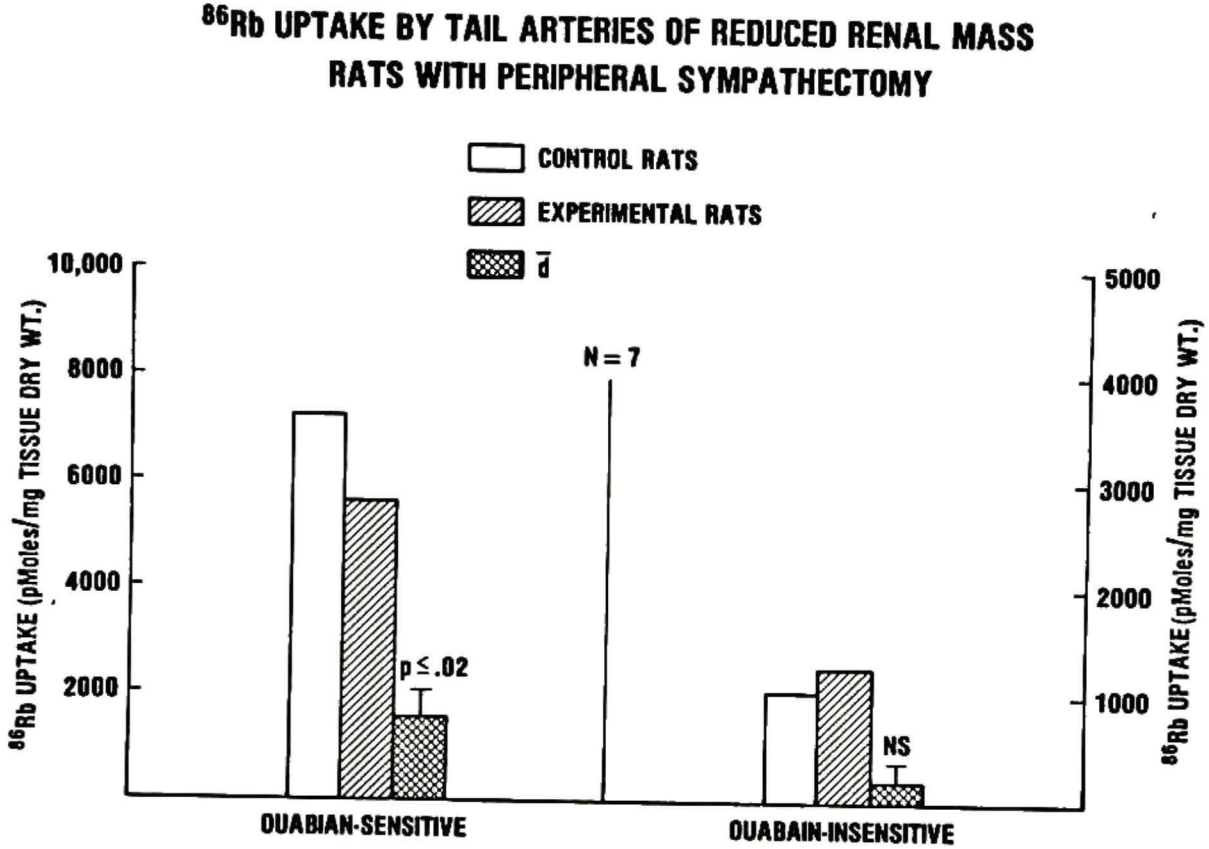
86Rb UPTAKE BY TAIL ARTERIES OF REDUCED RENAL MASS
RATS WITH PERIPHERAL SYMPATHECTOMY
CONTROL RATS
EXPERIMENTAL RATS
d̄
10,000
8000
6000
4000
2000
86Rb UPTAKE (pMoles/mg TISSUE DRY WT.)
5000
4000
3000
2000
1000
86Rb UPTAKE (pMoles/mg TISSUE DRY WT.)
N = 7
p ≤ .02
NS
OUABIAN-SENSITIVE
OUABAIN-INSENSITIVE

Figure 13

FIGURE 14. Plasma catecholamine (norepinephrine) levels of adrenal demedullated, peripherally sympathectomized, control normotensive and experimental hypertensive reduced renal mass rats.

PLASMA CATECHOLAMINE LEVELS OF REDUCED RENAL MASS RATS WITH PERIPHERAL SYMPATHECTOMY

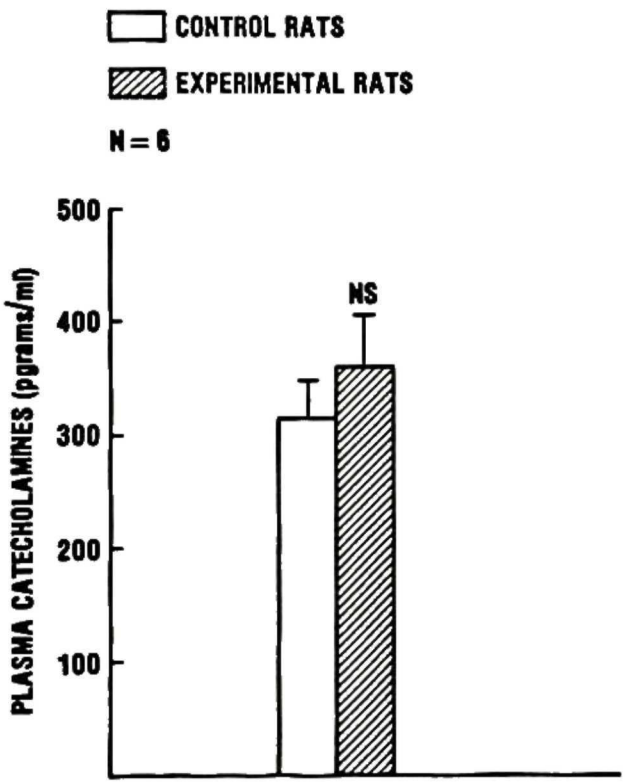

Figure 14

FIGURE 15. Average weekly body weights of centrally sympathectomized, control and experimental rats before and after intraventricular injection of 6-hydroxydopamine and for five weeks after subtotal nephrectomy.

BODY WEIGHTS OF REDUCED RENAL MASS RATS WITH CENTRAL SYMPATHECTOMY

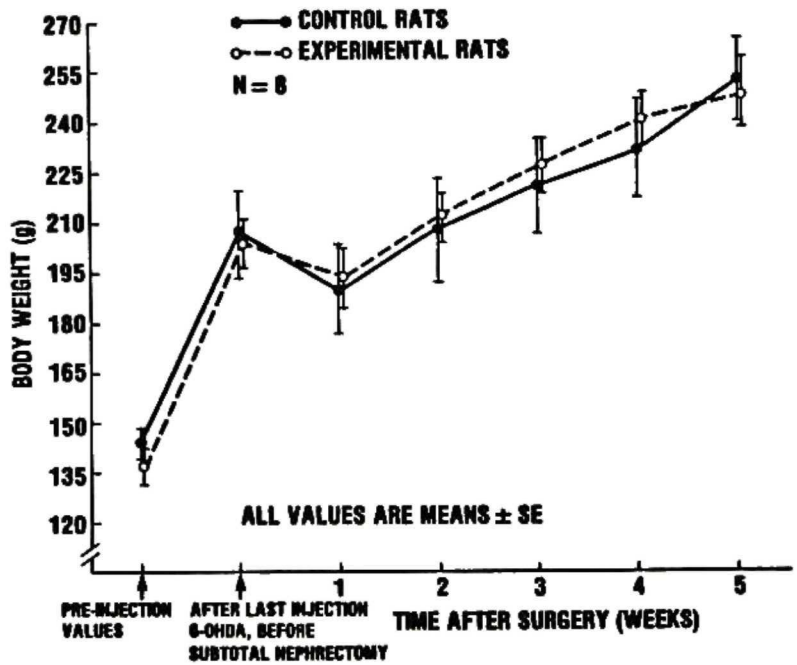

Figure 15

FIGURE 16. Average weekly systolic blood pressures (tail plethysmography) of centrally sympathectomized control and experimental rats before and after intraventricular injection of 6-hydroxydopamine and for five weeks after subtotal nephrectomy.

SYSTOLIC BLOOD PRESSURES (TAIL PLETHYSMOGRAPHY) OF REDUCED RENAL MASS RATS WITH CENTRAL SYMPATHECTOMY

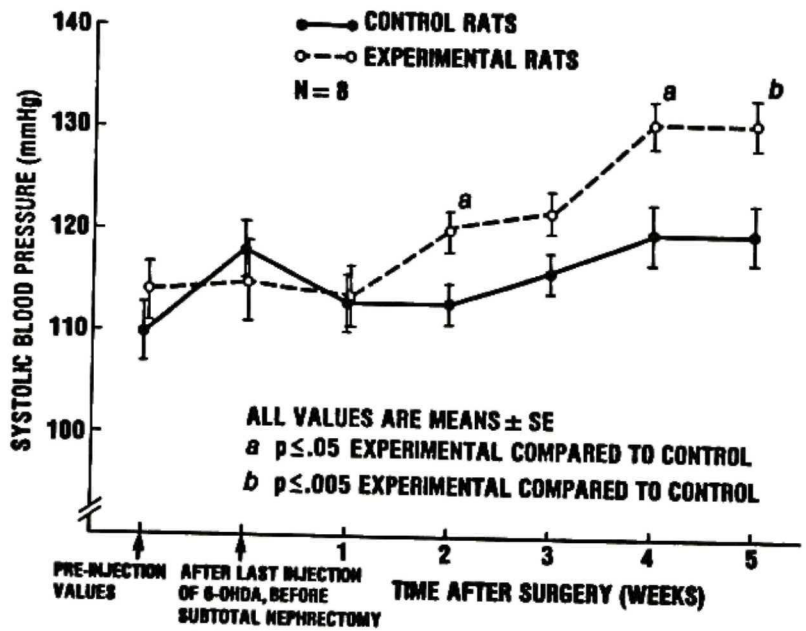

Figure 16

FIGURE 17. Ouabain-sensitive and ouabain-insensitive ^{86}Rb uptake by tail arteries from centrally sympathectomized control normotensive and experimental normotensive reduced renal mass rats.

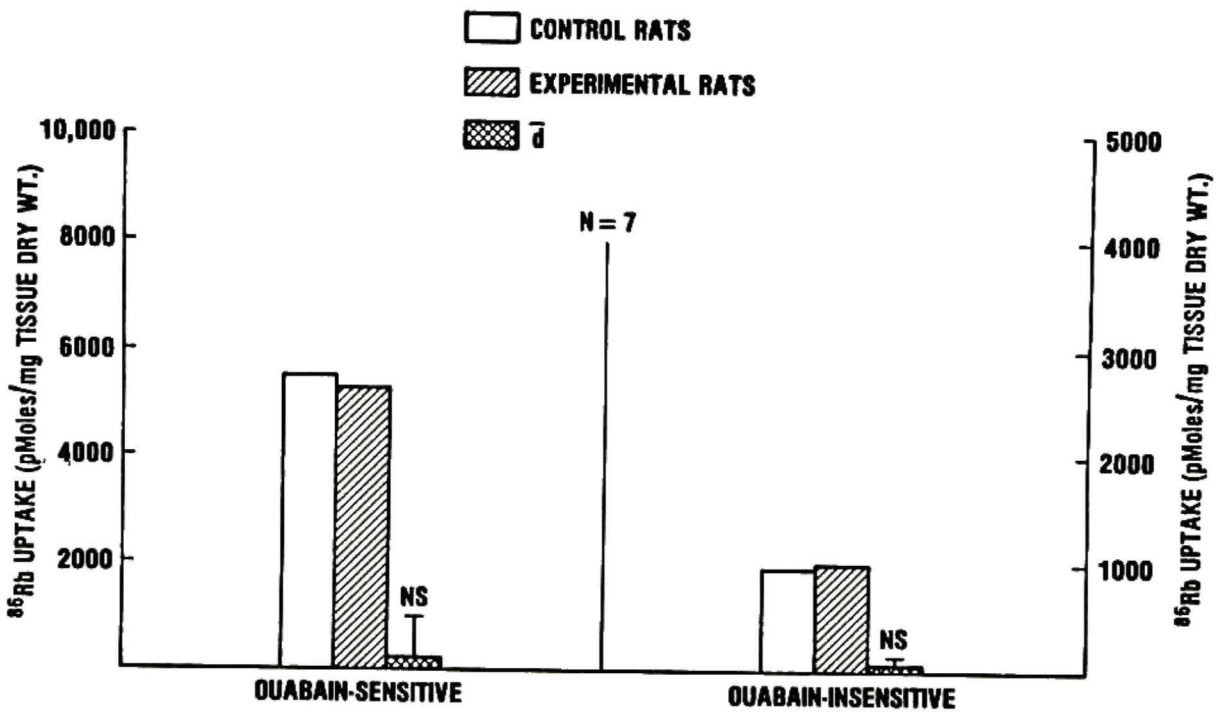
^{86}Rb UPTAKES BY TAIL ARTERIES FROM REDUCED RENAL MASS RATS WITH CENTRAL SYMPATHECTOMY
CONTROL RATS
EXPERIMENTAL RATS
$\bar{d}$
^{86}Rb UPTAKE (pMoles/mg TISSUE DRY WT.)
10,000
8000
6000
4000
2000
5000
4000
3000
2000
1000
N = 7
NS
NS
OUABAIN-SENSITIVE
OUABAIN-INSENSITIVE

Figure 17

FIGURE 18. Total ^{86}Rb uptake by tail arteries from normal untouched rats incubated in supernates prepared from boiled plasma of centrally sympathectomized control normotensive and experimental normotensive reduced renal mass rats.

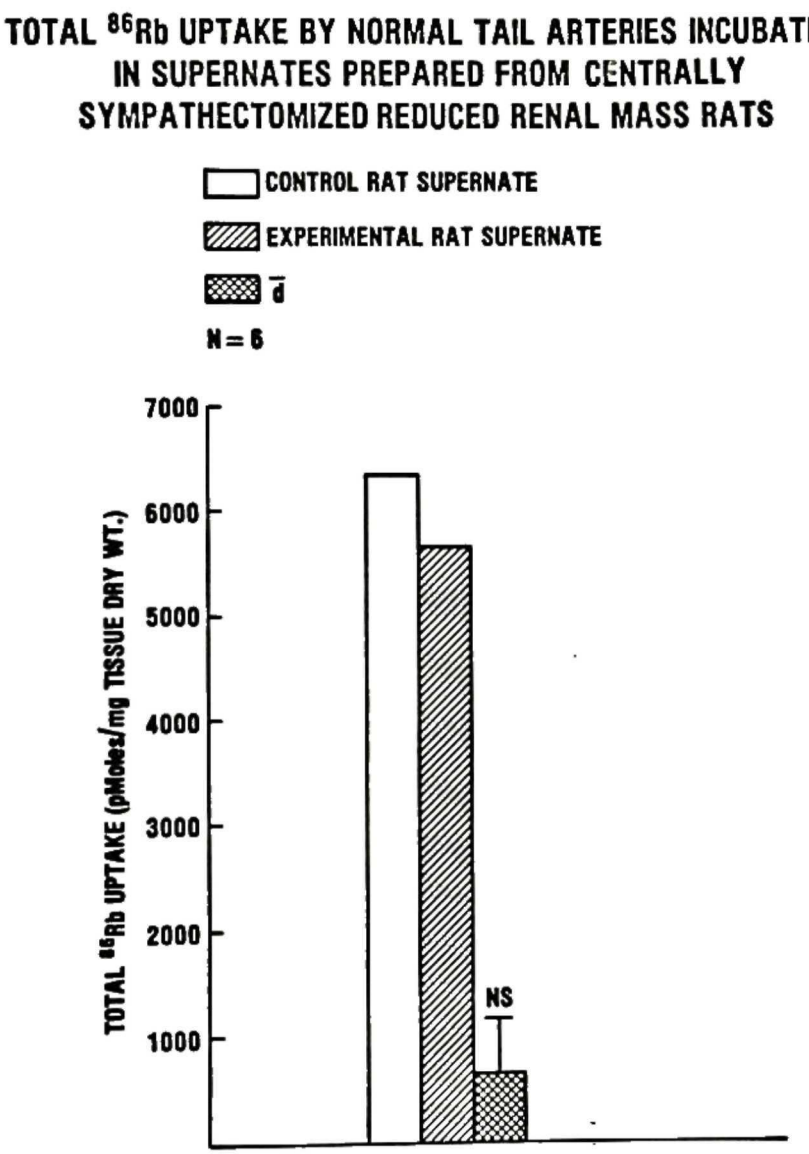
TOTAL 86Rb UPTAKE BY NORMAL TAIL ARTERIES INCUBATED IN SUPERNATES PREPARED FROM CENTRALLY SYMPATHECTOMIZED REDUCED RENAL MASS RATS
CONTROL RAT SUPERNATE
EXPERIMENTAL RAT SUPERNATE
d̄
N = 6
TOTAL 86Rb UPTAKE (pMoles/mg TISSUE DRY WT.)
7000
6000
5000
4000
3000
2000
1000
NS

Figure 18

Figures 15 and 16 present the weekly body weights and systolic blood pressures, respectively of control (distilled water drinking) and experimental (saline drinking) reduced renal mass rats with central sympathectomy. Body weights of the control and experimental rats were not significantly different at any time during this study. Systolic blood pressures of the control and experimental rats were not significantly different before or after the last injection of 6-hydroxydopamine. Although the average systolic blood pressure of the experimental rats was significantly higher than that of the control rats at weeks two, four, and five following subtotal nephrectomy, systolic blood pressure of the experimental rats did not reach hypertensive levels (140 mmHg) at any time during this study.

Furthermore, neither ouabain-sensitive nor ouabain-insensitive ^{86}Rb uptakes were significantly different in tail arteries from the control compared to experimental rats (Figure 17).

Additionally, total ^{86}Rb uptakes were not significantly different by tail arteries from normal rats incubated in boiled supernates obtained from centrally sympathectomized, subtotally nephrectomized, experimental and control rats (Figure 18).

The data presented in Table 5 shows that both norepinephrine and dopamine levels were significantly lower in brains from centrally sympathectomized control and experimental rats compared to levels in brains from normal untouched rats. The levels of these catecholamines in control and experimental rats were not different.

Acute <u>in vitro</u> sympathectomy (by treatment with 6-hydroxydopamine) of tail arteries obtained from control normotensive and paired experimental hypertensive rats did not change the percent inhibition of

TABLE 5. Group means ± SE of whole brain catecholamine levels in normal untouched rats and centrally sympathectomized control normotensive and experimental hypertensive reduced renal mass rats.

Table 5

WHOLE BRAIN CATECHOLAMINE LEVELS IN CENTRALLY SYMPATHECTOMIZED REDUCED RENAL MASS RATS

	NOREPINEPHRINE (nMoles/g BRAIN)	DOPAMINE (nMoles/g BRAIN)
UNTOUCHED RATS N = 3	3.94 ± .21	7.06 ± .59
CONTROL RATS N = 3	1.29 ± .21$^{\alpha}$	1.31 ± .59$^{\alpha}$
EXPERIMENTAL RATS N = 3	0.99 ± .21$^{\alpha}$	1.37 ± .59$^{\alpha}$

α $p \leq .05$ COMPARED TO UNTOUCHED RATS

ouabain-sensitive ^{86}Rb uptake by vessels from hypertensive rats compared to controls and ouabain-insensitive uptakes again were not different (Figure 19). Ouabain-sensitive ^{86}Rb uptake was however, decreased 50 percent by the acute in vitro denervation, which is consistent with findings by other investigators that denervation decreases Na^+-K^+ pump activity (Figure 20).

The change in absorbance of the 6-hydroxydopamine solutions during the 10 minute tail artery incubation averaged 0.07 ± .05 absorbance units. Changes in absorbance of 0.08 units or less are indicative of minimum oxidation of 6-hydroxydopamine (Aprigliano and Hermsmyer, 1976).

ROLE OF THE AV3V REGION IN REDUCED RENAL MASS HYPERTENSION

Results from experiments with AV3V lesioned and sham lesioned reduced renal mass rats were similar to those obtained with centrally sympathectomized reduced renal mass rats.

Table 6 presents general data on rats used in this study. Before AV3V lesioning or sham-lesioning, body weights of the two groups of rats were not different. AV3V lesioned rats showed a significant decrease in fluid intake and body weight following the AV3V lesioning procedure. In contrast, sham-lesioned rats showed no such decrease in fluid intake or body weight. Three weeks after lesioning fluid intake of lesioned and sham-lesioned rats were not different, indicating complete fluid intake recovery by the lesioned rats. In spite of complete fluid intake recovery the AV3V lesioned rats weighed significantly less than the sham-lesioned rats due to the initial decrease in fluid intake and body weight which immediately followed the lesioning procedure.

Figures 21 and 22 show the average weekly body weights and systolic blood pressures, respectively of the subtotally nephrectomized

FIGURE 19. Ouabain-sensitive and ouabain-insensitive ^{86}Rb uptakes by tail arteries from control normotensive and experimental hypertensive reduced renal mass rats, with *in vitro* sympathectomy by 6-hydroxydopamine plus phentolamine.

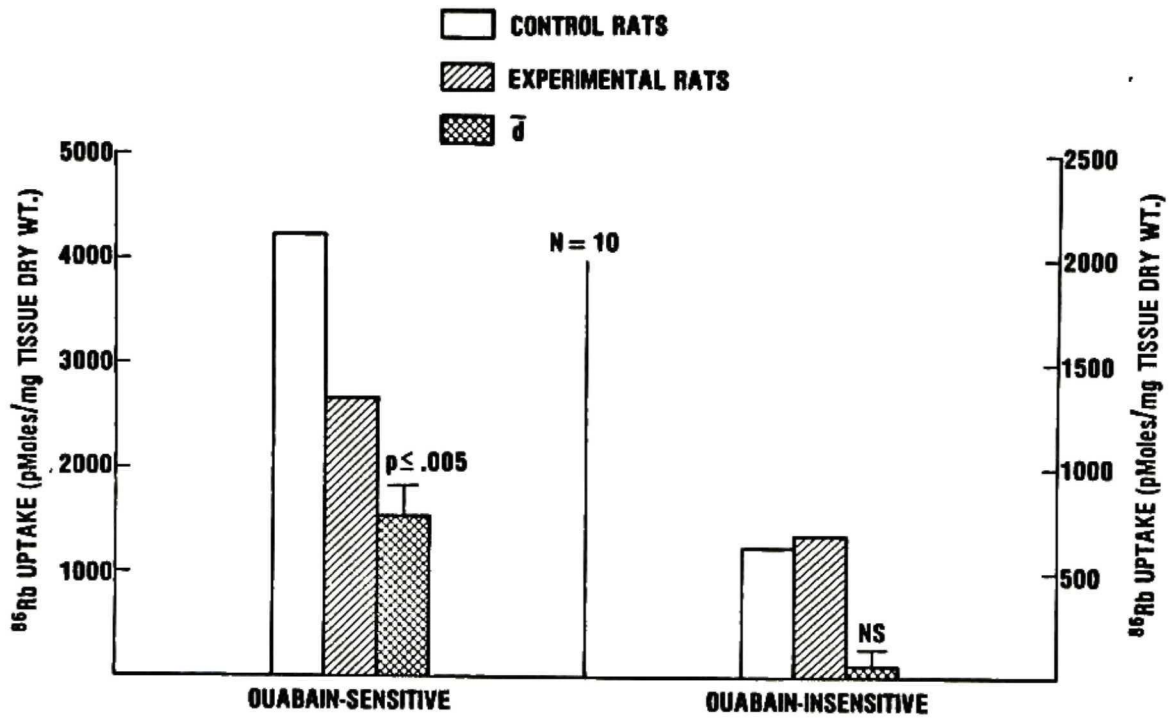
^{86}Rb UPTAKE BY TAIL ARTERIES FROM REDUCED RENAL MASS RATS AFTER IN VITRO SYMPATHECTOMY
CONTROL RATS
EXPERIMENTAL RATS
$\bar{d}$
5000
4000
3000
2000
1000
2500
2000
1500
1000
500
^{86}Rb UPTAKE (pMoles/mg TISSUE DRY WT.)
^{86}Rb UPTAKE (pMoles/mg TISSUE DRY WT.)
N = 10
p≤ .005
NS
OUABAIN-SENSITIVE
OUABAIN-INSENSITIVE

Figure 19

FIGURE 20. Ouabain-sensitive ^{86}Rb uptake by tail arteries from control normotensive and experimental hypertensive reduced renal mass rats with and without *in vitro* sympathectomy by 6-hydroxydopamine plus phentolamine.

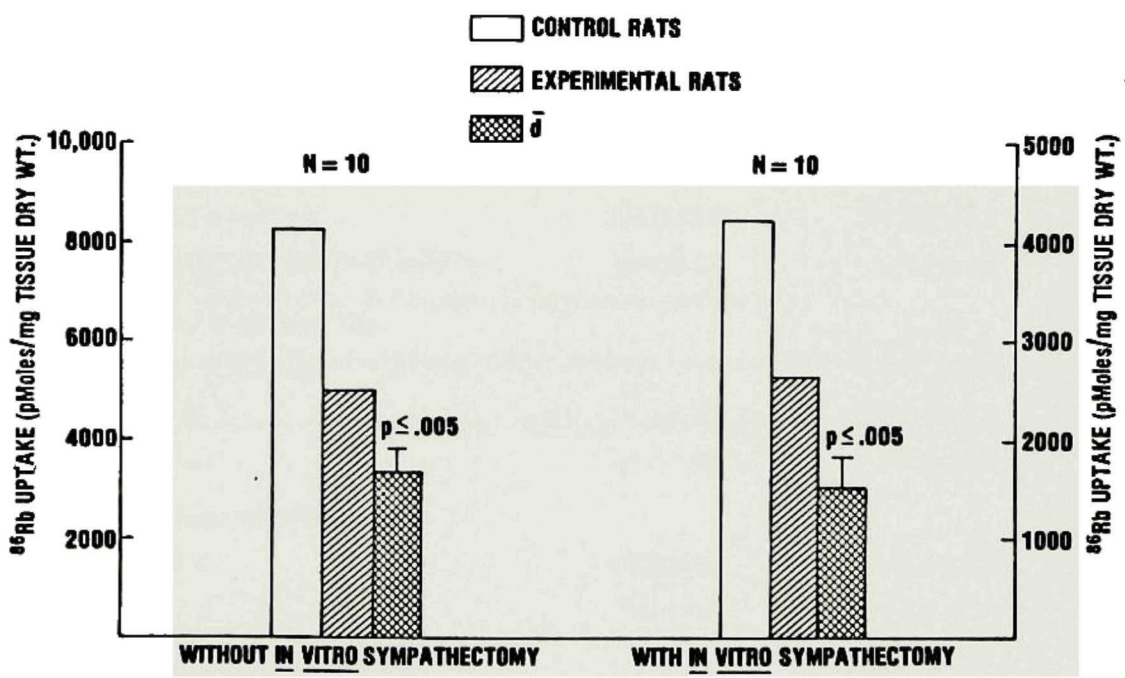
OUABAIN-SENSITIVE 86Rb UPTAKE BY TAIL ARTERIES FROM REDUCED RENAL MASS RATS
CONTROL RATS
EXPERIMENTAL RATS
d̄
86Rb UPTAKE (pMoles/mg TISSUE DRY WT.)
10,000
8000
6000
4000
2000
N = 10
p ≤ .005
5000
4000
3000
2000
1000
N = 10
p ≤ .005
86Rb UPTAKE (pMoles/mg TISSUE DRY WT.)
WITHOUT IN VITRO SYMPATHECTOMY
WITH IN VITRO SYMPATHECTOMY

Figure 20

TABLE 6. Group means ± SE of body weights and daily fluid intakes of sham and AV3V-lesioned rats before lesioning, after lesioning, and after complete hydration recovery.

Table 6

GENERAL DATA AV3V LESIONED AND SHAM LESIONED RATS

	AV3V LESIONED RATS (N = 5)	SHAM LESIONED RATS (N = 5)
BEFORE AV3V OR SHAM LESION		
BODY WEIGHT (g)	215.2 ± 8.3	198.5 ± 7.7
AVERAGE FLUID INTAKE (ml/DAY)	34.0 ± 4.5	30.5 ± 1.5
AFTER AV3V OR SHAM LESION		
BODY WEIGHT (g)		
DAY 1	181.0 ± 4.1	192.3 ± 7.1
DAY 2	165.7 ± 4.7	197.3 ± 8.2
AVERAGE FLUID INTAKE (ml/DAY)		
DAY 1	10.2 ± 2.3	29.0 ± 2.5
DAY 2	4.8 ± 1.2	27.8 ± 2.2
AFTER HYDRATION RECOVERY		
BODY WEIGHT (g)	184.5 ± 6.5	231.2 ± 9.9
AVERAGE FLUID INTAKE (ml/DAY)	34.2 ± 5.9	31.1 ± 2.3

FIGURE 21. Average weekly body weights of sham-lesioned and AV3V lesioned rats before and after the lesioning procedure and for five weeks after subtotal nephrectomy.

BODY WEIGHTS OF AV3V LESIONED AND SHAM LESIONED REDUCED RENAL MASS RATS

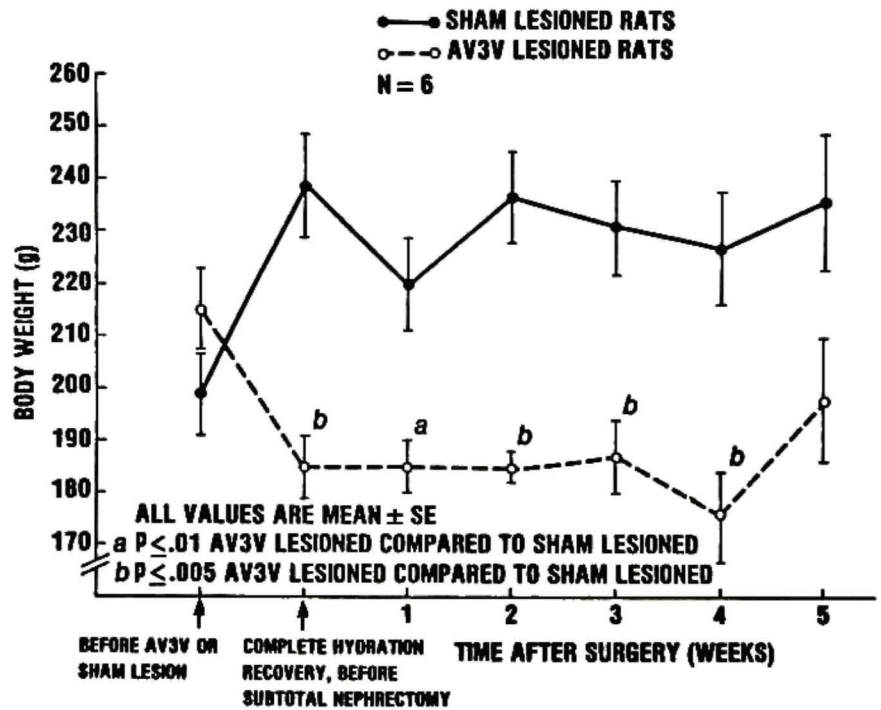

Figure 21

FIGURE 22. Average weekly systolic blood pressures (tail plethysmography) of sham-lesioned and AV3V lesioned rats before and after the lesioning procedure and for five weeks after subtotal nephrectomy.

SYSTOLIC BLOOD PRESSURES (TAIL PLETHYSMOGRAPHY) OF AV3V LESIONED AND SHAM LESIONED REDUCED RENAL MASS RATS

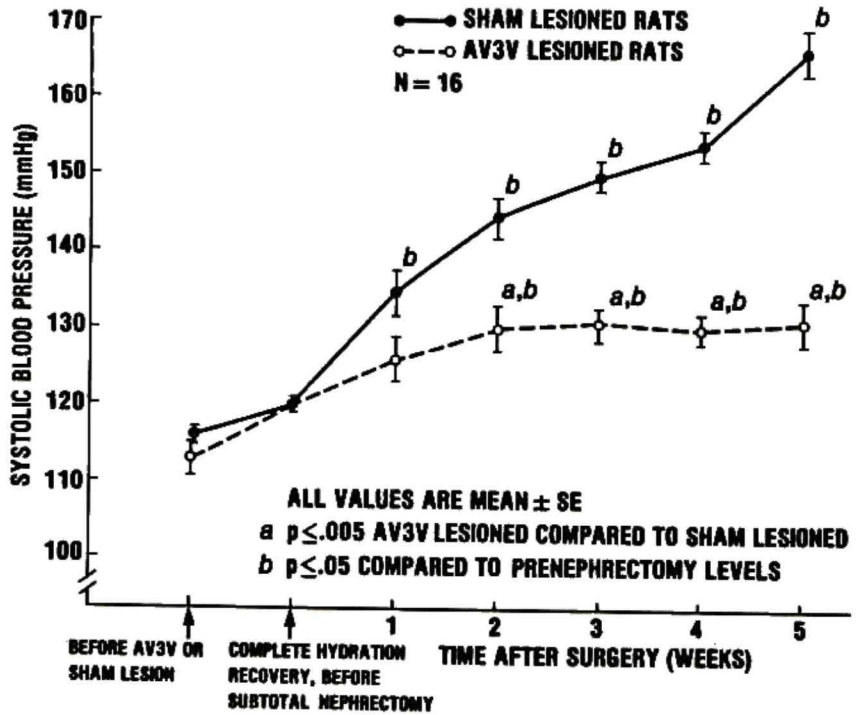

Figure 22

AV3V lesioned (saline drinking) and sham lesioned (saline drinking) rats. Body weights did not significantly change in either group following subtotal nephrectomy, and body weights of the sham-lesioned rats remained significantly greater than the AV3V lesioned rats from the time of lesioning throughout the duration of the study. Systolic blood pressures of the sham-lesioned rats increased progressively following subtotal nephrectomy and had reached hypertensive levels (systolic blood pressure > 140 mmHg) by the second week following subtotal nephrectomy.

By two weeks after subtotal nephrectomy systolic blood pressures of the AV3V lesioned rats were significantly higher than pre-nephrectomy levels, but at no time during this study did their systolic blood pressures reach the hypertensive level of 140 mmHg. Furthermore, following subtotal nephrectomy systolic blood pressure of the sham lesioned rats was significantly higher than the AV3V lesioned rats at all times.

Figure 23 is a representative photograph of the region destroyed by the AV3V lesioning procedure. The lesion is limited to the periventricular tissue in the most anterior and ventral portion of the third ventricle. The anatomical structures destroyed by this lesion include preoptic periventricular nuclei, anterior hypothalamic nuclei, the median preoptic nucleus, and the anterior wall of the third ventricle with the associated organum vasculosum of the lamina terminalis.

Measurement of ouabain-sensitive and insensitive ^{86}Rb uptakes by tail arteries from sham lesioned and AV3V lesioned reduced renal mass rats are shown in Figure 24. Ouabain-sensitive ^{86}Rb uptake by tail arteries was lower in sham lesioned rats in five of the six pairs of rats studied, but this was not a statistically significant difference, possibly because of the small sample size. Ouabain-insensitive uptakes were

FIGURE 23. Photograph of the region lesioned in the AV3V rats. Abbreviations: AC = anterior commisure; IIIV = third ventricle; MT = mamillo-thalamic tract; PVN = paraventricular nucleus; OVLT = organum vasculosum of the lamina terminalis; OC = optic chiasm; SCN = suprachiasmatic nucleus. The photos are enlarged 8.25 times the actual size. Photos are nissl-stained horizontal sections (40 u thick). Panel A is at the level of the anterior commisure, panel B is at the level of the paraventricular nucleus, panel C is at the level of the OVLT, panel D is at the optic chiasm. The * represents the center of the lesion in each panel with the arrows pointing to the margins of the lesion.

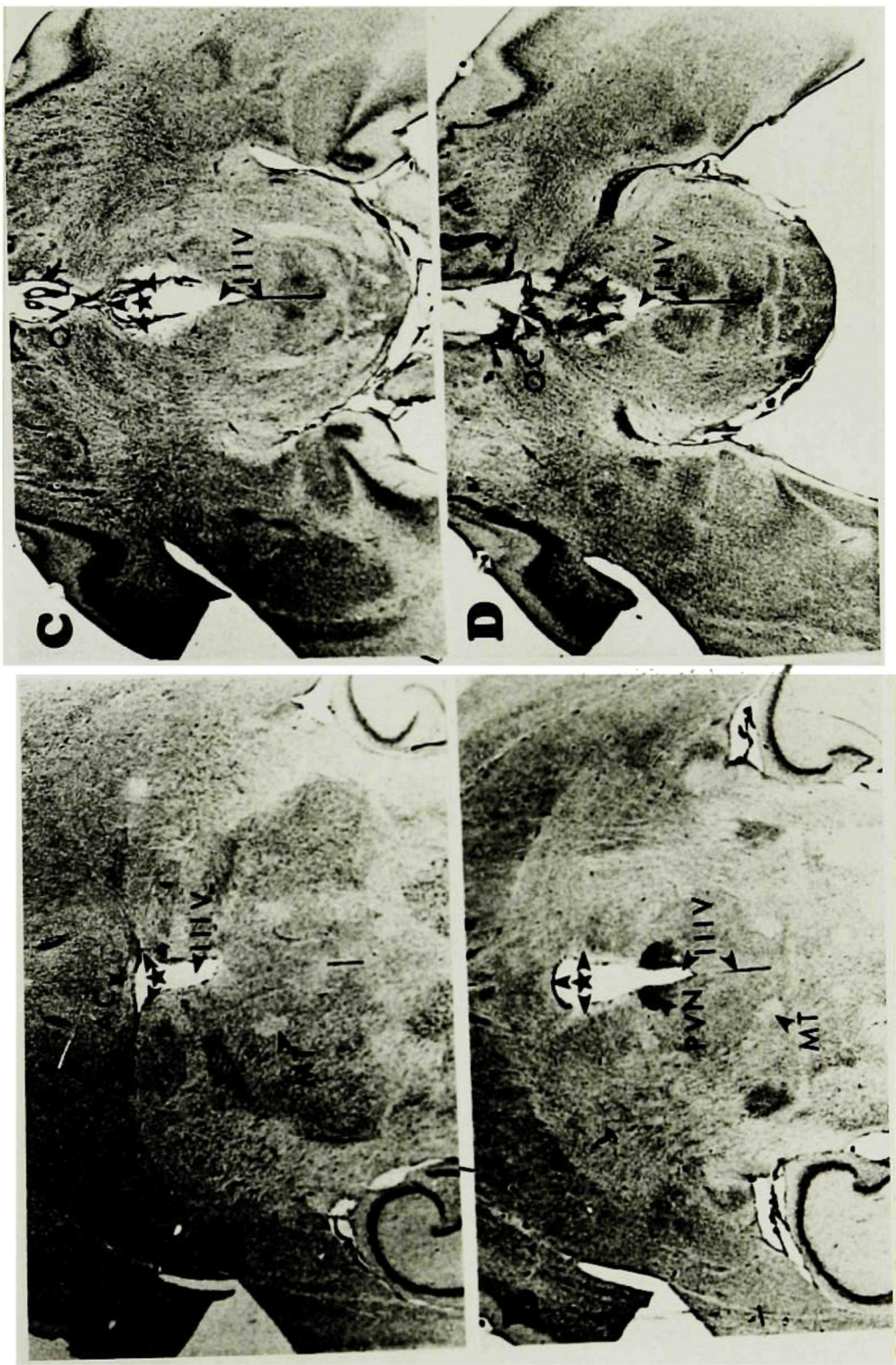

Figure 23

FIGURE 24. Ouabain-sensitive and ouabain-insensitive ^{86}Rb uptakes by tail arteries from sham-lesioned hypertensive and AV3V lesioned normotensive reduced renal mass rats.

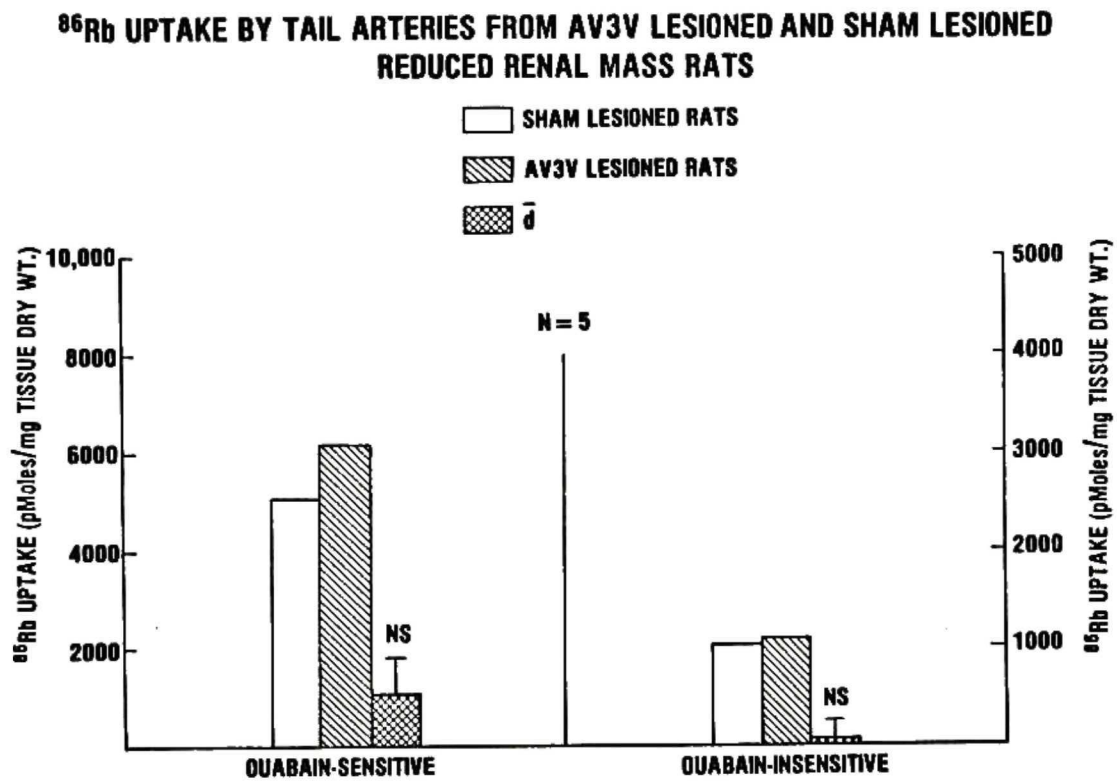
^{86}Rb UPTAKE BY TAIL ARTERIES FROM AV3V LESIONED AND SHAM LESIONED REDUCED RENAL MASS RATS
SHAM LESIONED RATS
AV3V LESIONED RATS
$\bar{d}$
^{86}Rb UPTAKE (pMoles/mg TISSUE DRY WT.)
10,000
8000
6000
4000
2000
5000
4000
3000
2000
1000
N = 5
NS
NS
OUABAIN-SENSITIVE
OUABAIN-INSENSITIVE

Figure 24

FIGURE 25. Total ^{86}Rb uptake by tail arteries from normal untouched rats incubated in supernates prepared from boiled plasma of sham-lesioned hypertensive and AV3V lesioned normotensive reduced renal mass rats.

TOTAL ^{86}Rb UPTAKE BY NORMAL TAIL ARTERIES INCUBATED IN SUPERNATES PREPARED FROM AV3V LESIONED AND SHAM LESIONED REDUCED RENAL MASS RATS

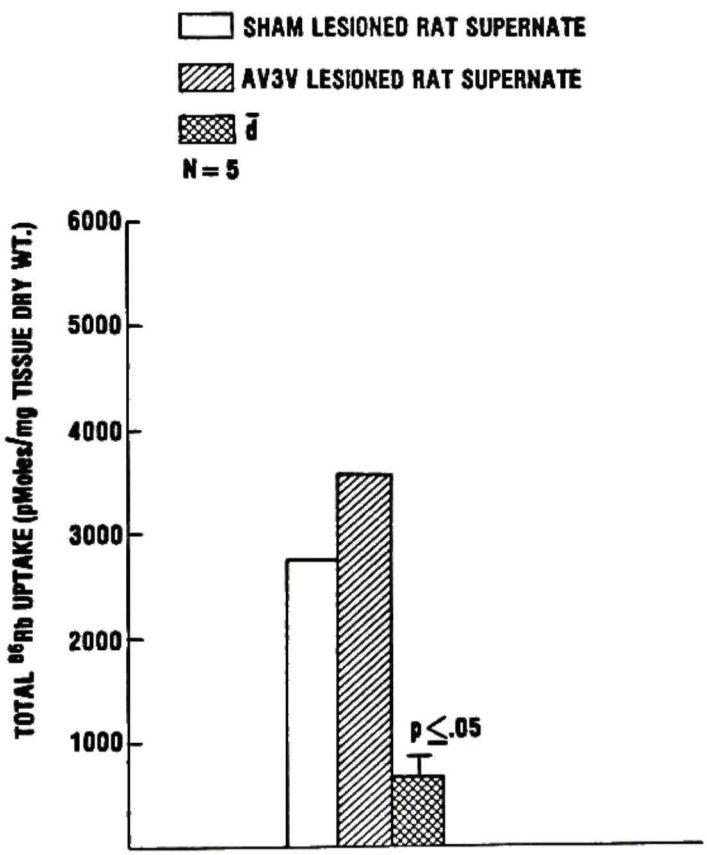

Figure 25

not significantly different.

However, incubation of normal rat tail arteries in supernates prepared from boiled plasma of sham lesioned (hypertensive) rats depressed ^{86}Rb uptake compared to arteries incubated in AV3V lesioned (normotensive) rat supernates (Figure 25).

PLASMA RENIN ACTIVITY, PLASMA VOLUMES, AND EXTRACELLULAR FLUID VOLUMES OF REDUCED RENAL MASS RATS

Figure 26 and Table 7 present data obtained from measurement of plasma renin activity and plasma as well as extracellular fluid volumes, respectively of control normotensive and experimental hypertensive rats. The experimental rats had significantly lower plasma renin activity than the control rats. Although absolute plasma volumes were not significantly different in the control and experimental rats, relative plasma volumes were significantly greater in the experimental rats compared to the controls. Results from both sodium thiocyanate and ^{14}C-inulin methods indicate that absolute as well as relative extracelluar fluid volumes were significantly greater in experimental hypertensive rats compared to control normotensive rats.

SERUM COMPOSITION OF REDUCED RENAL MASS RATS

Shown in Table 8 is the serum composition of control normotensive and experimental hypertensive reduced renal mass rats. Serum Na^+, K^+, osmolality, creatinine, and Ca^{2+} values were not different in control and experimental rats. The serum chloride level was significantly higher in the experimental rats while serum protein and Mg^{2+} levels were significantly higher in the control rats.

FIGURE 26. Plasma renin activity of control normotensive and experimental hypertensive reduced renal mass rats.

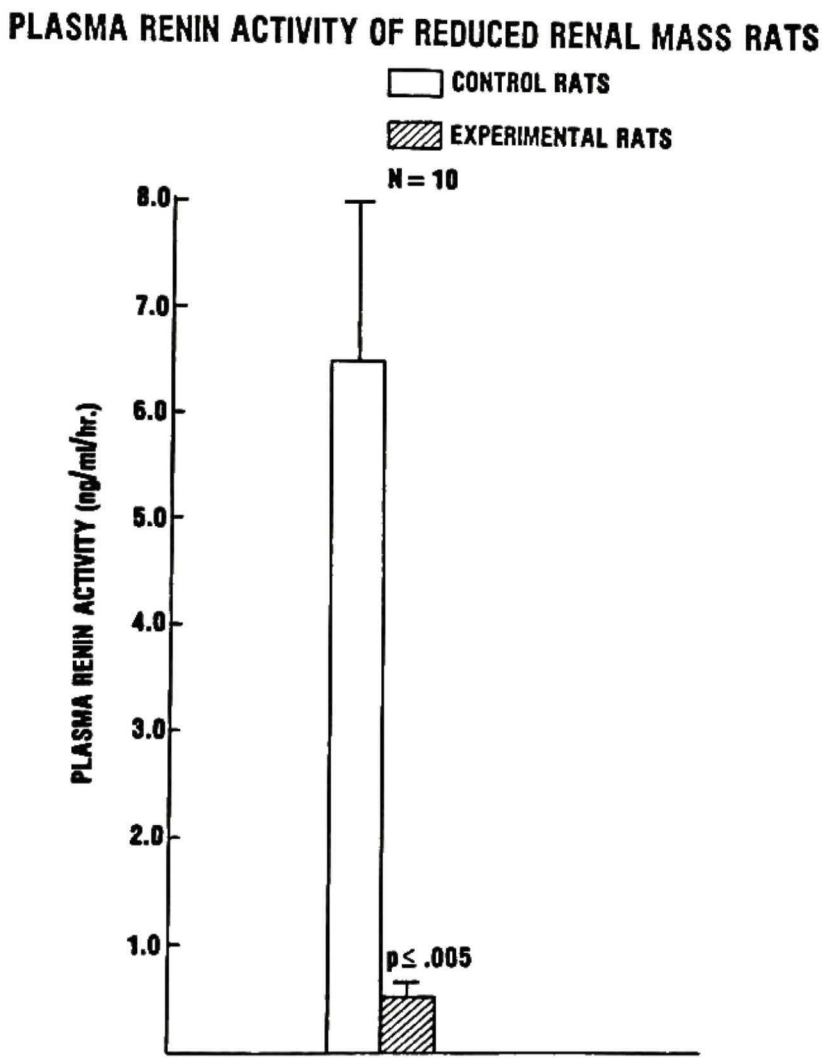
PLASMA RENIN ACTIVITY OF REDUCED RENAL MASS RATS
CONTROL RATS
EXPERIMENTAL RATS
N = 10
PLASMA RENIN ACTIVITY (ng/ml/hr.)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
p≤ .005

Figure 26

TABLE 7. Group means ± SE of absolute (ml) and relative (ml/Kg body wt.) plasma and extracellular fluid volumes of control normotensive and experimental hypertensive reduced renal mass rats.

Table 7

SERUM COMPOSITION OF REDUCED RENAL MASS RATS FIVE WEEKS AFTER SURGERY

	CONTROL RATS	EXPERIMENTAL RATS	
Na^+ (mEq/l)	147.3 ± 0.8	150.1 ± 1.8	N = 20
K^+ (mEq/l)	5.0 ± 0.1	4.8 ± 0.1	N = 20
Cl^- (mEq/l)	112.4 ± 1.0[b]	117.9 ± 1.9	N = 20
OSMOLALITY (mOsm/kg)	341.2 ± 2.4	338.8 ± 4.3	N = 20
CREATININE (mg%)	1.05 ± 0.23	1.06 ± 0.05	N = 20
BUN (mg%)	48.8 ± 2.4[c]	37.5 ± 2.5	N = 20
PROTEIN (g%)	6.5 ± 0.2[c]	5.8 ± 0.2	N = 20
Ca^{2+} (mEq/l)	5.9 ± 0.1	5.9 ± 0.1	N = 20
Mg^{2+} (mEq/l)	2.06 ± 0.10[a]	1.53 ± 0.16	N = 20

a $p \leq .01$, *b* $p \leq .025$, *c* $p \leq .005$

TABLE 8. Group means ± SE of serum composition from control normotensive and experimental hypertensive reduced renal mass rats.

Table 8

PLASMA AND EXTRACELLULAR FLUID VOLUMES OF REDUCED RENAL MASS RATS

	CONTROL RATS	EXPERIMENTAL RATS
PLASMA VOLUME (EVANS BLUE)		
ABSOLUTE (ml) N = 9	11.49 ± .93	13.00 ± .99
RELATIVE (ml/kg BODY WT.) N = 9	37.26 ± 2.11	50.64 ± 4.22[b]
EXTRACELLULAR FLUID VOLUME (SODIUM THIOCYANATE)		
ABSOLUTE (ml) N = 9	57.24 ± 3.25	73.59 ± 2.10[c]
RELATIVE (ml/kg BODY WT.) N = 9	187.03 ± 5.02	287.70 ± 11.41[c]
(^{14}C INULIN)		
ABSOLUTE (ml) N = 3	64.41 ± 3.51	83.73 ± 6.25[a]
RELATIVE (ml/kg BODY WT.) N = 3	200.72 ± 8.49	264.21 ± 9.46[c]

a $p \leq .05$ EXPERIMENTAL COMPARED TO CONTROL

b $p \leq .025$ EXPERIMENTAL COMPARED TO CONTROL

c $p \leq .005$ EXPERIMENTAL COMPARED TO CONTROL

DISCUSSION

REDUCED RENAL MASS HYPERTENSION MODEL

Our findings indicate that in subtotally nephrectomized rats (70 to 80 percent renal mass removed) increasing the sodium intake by substitution of a one percent NaCl solution for drinking induces the development of hypertension whereas restriction of sodium intake (low sodium chow with distilled water for drinking) prevents the development of hypertension. Similar findings have been reported by other laboratories (Espinel, 1976; Ylitalo et al., 1976; Pitcock et al., 1980).

Our measurements of plasma renin activity (PRA) and of plasma as well as extracellular fluid volumes (ECFV) in these subtotally nephrectomized normotensive control (distilled water drinking) and hypertensive experimental (saline drinking) rats indicate that the reduced renal mass model of hypertension is a low renin, volume-expanded model of hypertension. These results are supported by the findings of Ylitalo and Gross (1979) and Pitcock et al. (1980). Our PRA values in saline-drinking reduced renal mass hypertensive rats are similar to those reported by Ylitalo and Gross (1979). Additionally, Ylitalo and Gross (1979) and Pitcock et al. (1980) also reported similar increases in plasma and extracellular fluid volumes in these hypertensive rats.

Na^+-K^+ PUMP ACTIVITY

Our findings indicate that compared to tail arteries from subtotally nephrectomized mormotensive rats, ouabain-sensitive ^{86}Rb uptake was decreased in tail arteries from subtotally nephrectomized hypertensive rats. No such difference was seen in ouabain-insensitive uptake between arteries from hypertensive and normotensive animals. Because

rubidium substitutes for potassium in active transport by the Na^+-K^+ pump (see background), these results indicate that there may be an inhibition of Na^+-K^+ pump activity in the vascular smooth muscle cells of rats with reduced renal mass hypertension. Additionally, our finding of decreased Na^+, K^+-ATPase activity in microsomes prepared from the myocardium of hypertensive compared to normotensive rats indicates that Na^+-K^+ pump activity is inhibited both in vascular as well as cardiac muscle cells.

Our finding of increased Mg^{2+}-ATPase activity in the myocardial microsomes prepared from the hypertensive rats is intriguing. This has been a consistent finding in the myocardium obtained from rats with one-kidney, one clip and one-kidney, DOCA, salt hypertension (Clough et al., 1977; 1978). Although there is some speculation that plasma membrane Mg^{2+}-ATPase activity may have a role in the regulation of facilitated diffusion of certain substances (Mircevova, 1977), no definite function has been ascribed to this enzyme activity. Also, the Mg^{2+}-ATPase activity observed here is probably due to the presence of a heterogeneous group of enzymes from various types of membranes. We therefore cannot speculate on the physiolological significance of this finding.

Since Na^+, K^+-ATPase activity is expressed as uMoles Pi/mg protein/hour (Figure 6), it could be argued that our finding of decreased Na^+, K^+-ATPase activity was due to a decrease in the plasma membrane to total protein ratio in microsomes prepared from myocardium of the hypertensive rats (these microsomes also contain proteins other than plasma membranes). This could occur secondary to hypertrophy which is evident in myocardium of hypertensive rats. Hypertrophy might alter the sedimentation properties of plasma membranes or decrease the cellular

surface area to volume ratio. Either of these effects could possibly alter the plasma membrane to total protein ratio. However, this does not appear to be the case since the content of sialic acid, a plasma membrane marker (Jones et al., 1978), was not different in the microsomes prepared from the hypertensive and control rats (Figure 6).

Our finding of decreased Na^+-K^+ pump activity in tail arteries of RRM HT rats compared to tail arteries from RRM NT rats in spite of *in vitro* sympathetic denervation of these vessels by 6-hydroxydopamine further indicates that the observed pump suppression is not due to altered local sympathetic nerve activity.

The finding of decreased cardiovascular muscle cell Na^+-K^+ pump activity in reduced renal mass hypertensive rats is consistant with the findings in other low renin, investigator-induced models of experimental hypertension. Depressed Na^+-K^+ pump activity has been observed in tail arteries of rats with one-kidney, DOCA, salt hypertension (Pamnami et al., 1978a) and in mesenteric arteries, veins (Overbeck et al., 1976), and leucocytes (Pamnani et al., 1980b) of dogs with one-kidney, one wrapped hypertension. Decreased microsomal Na^+-K^+ ATPase activity has been observed in left ventricle of rats with one-kidney, DOCA, salt hypertension (Clough et al., 1978) and in left and right ventricle of rats with one-kidney, one clip hypertension (Clough et al., 1977a; 1977b).

Finding decreased Na^+-K^+ pump activity in mesenteric veins and in leucocytes of one-kidney, one wrapped hypertensive dogs (Pamnani et al., 1980b) and of decreased Na^+, K^+-ATPase activity in microsomes prepared from right ventricle of one-kidney, one clip hypertensive rats indicates that the observed impairment of Na^+-K^+ pump activity in these

models of hypertension is not secondary to elevated blood pressure.

Friedman and Nakashima (1976) however, using tail arteries from one-kidney, DOCA, salt hypertensive rats have reported increased vascular Na^+-K^+ pump activity (these investigators prepared their animals the same as was done by Pamnani et al.). Tail arteries were placed in a cold physiologic salt solution (PSS) in which lithium had been substituted for sodium. Compared to control rat arteries, lithium entered the hypertensive rat arteries more readily in exchange for sodium and potassium. From this they concluded that permeability of vascular smooth muscle cells is increased in arteries from the hypertensive animals. This finding is compatable with the finding of Pamnani et al. (1978a) who also reported increased ouabain-insensitive ^{86}Rb uptake by tail arteries from one-kidney, DOCA, salt hypertensive rats. Ouabain-insensitive ^{86}Rb uptake reflects distribution in extracellular space and passive penetration into cells which is dependent on surface area and permeability. Increased ouabain-insensitive ^{86}Rb uptake therefore suggests increased permeability. Thus in this respect the findings of the two groups are in agreement. However, Friedman and Nakashima did additional experiments in which they concluded that the pump compensates for this increased permeability with increased pump activity. This is clearly in contrast with the finding of Pamnani et al. (1978a).

The method used to measure Na^+-K^+ pump activity in these two laboratories is different and may account for the different results. The method used by Friedman and Nakashima involves perfusing the tail arteries for four and one-half hours before measurement of pump activity whereas Pamnami et al. measured pump activity in freshly excised vessels. As will be discussed later, there is reason to believe that

the observed suppression of cardiovascular muscle cell Na^+-K^+ pump activity in animals with some low renin, investigator-induced models of hypertension is due to a circulating ouabain-like agent. It is therefore possible that this agent is washed away or leached out when tissues are incubated in artificial media for prolonged periods of time. To study this possibility Pamnani et al. (1981a) repeated their experiments. However, this time two protocols were used. In the first, Na^+-K^+ pump activity was measured in freshly excised tail arteries and in the second, Na^+-K^+ pump activity was measured after the arteries had been incubated in a PSS for four and one-half hours at room temperature. With the freshly excised method Pamnani et al. confirmed their previous findings of decreased Na^+-K^+ pump activity and increased ouabain-insensitive ^{86}Rb uptake. However, when tissues were incubated for four and one-half hours in artificial PSS, the difference in ouabain-sensitive ^{86}Rb uptake by arteries from hypertensive and normotensive rats disappeared. In contrast, the ouabain-insensitive ^{86}Rb uptake (and therefore possibly permeability) was still significantly higher in arteries from hypertensive rats. These findings therefore strongly suggest that when tissues are kept <u>in vitro</u> in artificial media for prolonged periods of time certain changes occur. Possibly a factor which is responsible for the pump inhibition is washed away or is leached out. If this factor was washed away and cell membrane permeability remained increased, this would lead to increased pump activity due to stimulation by increased intracellular sodium or due to increased receptor synthesis that might have occured in response to chronic receptor blockade or to a direct effect of the DOCA (Trendelenburg, 1963; Gnegy and Costa, 1980).

Songu-Mize et al. (1981) have also recently reported decreased

Na^+-K^+ pump activity in freshly excised tail arteries of rats with one-kidney, DOCA, salt hypertension, thus confirming the findings of Pamnani et al.

These studies seem to indicate that suppressed cardiovascular muscle cell Na^+-K^+ pump activity is a common factor in low renin, investigator induced models of experimental hypertension.

That decreased Na^+-K^+ pump activity might play a role in hypertension is also supported by the observations of Edmondson et al. (1975), Thomas et al. (1975), and Edmondson and MacGregor (1981). Edmondson et al. showed that leucocyte sodium and water content were increased and active sodium efflux by leucocytes was decreased in essential hypertensive patients. Thomas et al. reported these same findings and further showed that these abnormalities were not present in patients with well-controlled hypertension (treated with methyldopa, adrenergic nerve blockers, or thiazide diuretics). Edmondson and MacGregor further showed that active sodium efflux by leucocytes from essential hypertensive patients was decreased most markedly in those patients with low plasma renin activity.

ROLE OF DEPRESSED Na^+-K^+ PUMP ACTIVITY IN HYPERTENSION

Since the Na^+-K^+ pump is electrogenic, pumping three Na^+ out of the cell for every two K^+ it pumps in (Thomas, 1972; Anderson, 1976), inhibition of pump activity would depolarize the membrane.

Hendricks and Casteels (1974) showed that addition of ouabain to the medium bathing an <u>in vitro</u> rabbit ear artery caused depolarization which was accompanied by increased tension. They concluded that the tension increase was brought about by an opening of voltage-dependent calcium channels which led to an increase in free intracellular

Ca^{2+} concentration.

There may also be a Na^+/Ca^{2+} exchange mechanism located on the plasma membranes of cardiovascular muscles. According to Blaustein (1977) this system normally functions to move three Na^+ into the cell in exchange for bringing one Ca^{2+} out. Na^+ moves down its electrochemical gradient, providing energy for the coupled movement of Ca^{2+} out of the cell against its electrochemical gradient. Blaustein has observed that in in vitro rabbit aorta strips ouabain causes increased tension. He concludes that inhibition of Na^+-K^+ pump activity by ouabain decreased the ratio of extracellular Na^+ to intracellular Na^+ and thereby inhibited extrusion of Ca^{2+} by the Na^+/Ca^{2+} exchange mechanism which then caused tension to increase. Blaustein further suggests that if the Na^+-K^+ pump is sufficiently inhibited, the ratio of extracellular Na^+ to intracellular sodium will decrease to such an extent that the Na^+/Ca^{2+} exchange mechanism will reverse and extrude Na^+ from the cell while bringing Ca^{2+} into the cell.

It is apparent then that decreased Na^+-K^+ pump activity could, via voltage-dependent Ca^{2+} channels or altered Na^+/Ca^{2+} exchange mechanism, cause vasoconstriction, increased blood vessel responsiveness to vasoactive agents, and increased cardiac contractility -- changes not unlike those seen in hypertension (see background).

Recently, Pamnani et al. (1981c) recorded membrane potentials and showed that vascular smooth muscle cells in tail arteries of rats with one-kidney, one clip hypertension were depolarized compared to vascular smooth muscle cells in tail arteries from one-kidney normotensive controls. This indicates that the observed pump suppression in these animals could (via voltage-dependent depolarization) enhance con-

tractility of the vascular smooth muscle cells and be responsible for the elevated blood pressure.

Na^+-K^+ PUMP ACTIVITY IN GENETIC MODELS OF HYPERTENSION

Decreased vascular Na^+-K^+ pump activity is not common to all models of experimental hypertension.

Friedman (1979) and Pamnani et al. (1979) showed that Na^+-K^+ pump activity was significantly higher in tail arteries of spontaneously hypertensive rats (SHR) compared to normotensive Wistar-Kyoto rats (WKY). Both laboratories also reported that permeability of the vessels from SHR appeared to be increased compared to WKY.

Similar findings have been reported in Dahl salt-sensitive (S) rats relative to Dahl salt-resistant (R) rats. Pamnani et al. (1980a) found increased permeability (ouabain-insensitive ^{86}Rb uptake) and Na^+-K^+ pump activity in tail arteries from high or low-salt-fed S rats compared to high or low-salt-fed R rats, respectively. Increased permeability and pump activity was also found in tail arteries of high-salt-fed S rats compared to low-salt-fed S rats and in high-salt-fed R rats compared to low-salt-fed R rats. Overbeck et al. (1981) have also found increased Na^+-K^+ pump activity in tail arteries of S rats compared to R rats at a high or low-sodium intake, but they did not find increased permeability. When the aortas from these rats were compared however, both Na^+-K^+ pump activity and permeability were increased in vessels from high-salt S rats compared to all the other groups.

This data suggests that the mechanism of elevated blood pressure in low renin, investigator-induced models of hypertension may be different from the genetically mediated (SHR and hypertensive S) models of hypertension.

TIME STUDY

Our finding of a temporal relationship between the suppression of vascular Na^+-K^+ pump activity in tail arteries of experimental (saline-drinking) reduced renal mass rats and the development of hypertension suggest that pump suppression may infact be involved in the development of this type of hypertension. Albeit we did not find a significant correlation between the percent of pump inhibition and the level of arterial blood pressure, this is quite understandable since regulation of blood pressure involves several other negative feedback mechanisms.

HUMORAL OUABAIN-LIKE FACTOR

Our finding of a circulating ouabain-like factor in rats with reduced renal mass hypertension suggests that this humoral inhibitor may be responsible for the suppressed cardiovascular muscle cell Na^+-K^+ pump activity seen in these animals.

This finding is again consistent with findings in animals with other low renin, investigator-induced models of experimental hypertension. Pamnani et al. (1980b and 1981b) have reported the presence of a ouabain-like factor in the plasma of rats with one-kidney, one clip hypertension and in the plasma of dogs with one-kidney, one wrappped hypertension. Additionally, Pamnani et al. (1981c) showed (<u>in vitro</u>) that this factor in the supernates of boiled plasma from one-kidney, one clip hypertensive rats depolarizes tail arteries from normotensive control rats to the same extent that ouabain does. Furthermore, in the presence of ouabain, hypertensive rat supernate had no additional effect on the membrane potential. These data indicate that the circulating ouabain-like factor found in some hypertensive animals can depolarize vascular smooth muscle cells and may explain the mechanism of their

elevated blood pressure. The circulation of this ouabain-like factor may explain why decreased Na^+-K^+ pump activity has been observed in almost all components of the cardiovascular system (myocardium, arteries, and veins). Also, Poston et al. (1981) have shown that active sodium transport of white cells and red cells obtained from patients with essential hypertension is inhibited and that incubation of leucocytes from normotensive subjects in serum obtained from hypertensive patients results in impaired active sodium transport by these leucocytes.

There are also several earlier studies which indicate that an "unknown" vasoactive agent is present in the plasma of animals with some types of experimental hypertension as well as in the plasma of some essential hypertensive humans.

Solandt et al. (1940) showed that cross-circulating blood between dogs with either one-kidney, one clip or one-kidney, one wrap hypertension and bilaterally nephrectomized assay dogs increases the blood pressure of the assay dogs. Dahl et al. (1969) showed that hypertension develops in salt-resistant R rats that are parabiotically joined to salt-sensitive S rats when both consume a high sodium diet. R rats by themselves do not become hypertensive when consuming a high sodium diet. Michelakis et al. (1974) showed that plasma of patients with malignant hypertension as well as plasma of dogs with one-kidney, one clip hypertension contains a sensitizing factor which increases blood vessel responsiveness to angiotensin II. Schmidt et al. (1974) showed that plasma from subtotally nephrectomized dogs consuming a diet containing 120 mEq Na^+/day contains a factor which causes natriuresis when injected into assay rats. However, when subtotally nephrectomized dogs were fed a low sodium diet this natriuretic factor could not be

found in their plasma. Self et al. (1976) showed that serum from hypertensive sodium-fed rats contains a sensitizing factor which increases blood vessel responsiveness to norepinephrine.

Collectively, these experiments strongly suggest the presence of a humoral factor(s) which may be involved in the pathophysiology of some types of hypertension. It is possible that the humoral agent responsible for the changes observed in these studies is the ouabain-like factor which we have found in hypertensive animals.

A study conducted by Pitcock et al. (1980) indicated that factors other than a circulating ouabain-like agent may play a role in the mechanism of the elevated blood pressure in reduced renal mass-saline hypertension. Pitcock et al. observed a significant decrease in the number and function of renomedullary interstial cells (RIC) in subtotally nephrectomized hypertensive rats. Subcutaneous implants of RIC have previously been shown to lower blood pressure of rats with one-kidney, one clip hypertension (Muirhead et al., 1977). Muirhead et al. suggest that RIC secrete a vasodilator substance which normally checks the action of vasoconstrictors in the homeostasis of blood pressure. Therefore, a decreased number or function of RIC would allow vasoconstrictor substances to exert a greater effect on blood pressure. Pitcock et al. (1980) reported that the degeneration of RIC they observed in reduced renal mass hypertensive rats appears to play a role in the pathogenesis of this hypertension. They concluded that a combination of the effects of sodium and volume and a renomedullary interstitial cell deficiency was responsible for the sustained hypertension in reduced renal mass saline-drinking rats.

Our study does not rule out the possibility that, in addition to

the circulaing ouabain-like factor, an RIC deficiency may also be involved in the mechanism of the elevated blood pressure in reduced renal mass hypertensive rats.

Our serum composition data from control normotensive and experimental hypertensive reduced renal mass rats indicate that both groups of animals were uremic (elevated blood urea nitrogen). Uremia itself is known to inhibit Na^+-K^+ pump activity through the action of substances such as methylguanidine. Welt (1969) for example showed that ouabain-inhibitable sodium efflux by erythrocytes from uremic patients was decreased. Edmondson et al. (1975) observed that leucocyte sodium and water content was increased in uremic patients with chronic renal failure. They further noted that the active sodium efflux by leucocytes from these patients was decreased compared to normal control subjects. In the present study, compared to controls, hypertensive rats were less uremic as indicated by their lower blood urea nitrogen levels. This was probably due to the higher renal perfusion pressure in the hypertensive rats. Since cardiovascular muscle cell Na^+-K^+ pump activity was lower in the hypertensive rats, uremia per se cannot account for the decreased pump activity observed in these animals.

FACTORS AFFECTING RELEASE OF THE HUMORAL AGENT

Peripheral Sympathetomy

The presence of decreased cardiovascular muscle cell Na^+-K^+ pump activity and the development of hypertension in peripherally sympathectomized, subtotally nephrectomized rats would suggest that the perepheral sympathectic system is not required for the development of this type of hypertension. However, normal rat plasma norepinephrine levels are 300 to 400 pg/ml (Avakian and Horvath, 1980; Shoup and Keefe,

1980) and plasma norepinephrine levels of our peripherally sympathectomized control and experimental rats were 314 ± 32 and 350 ± 44 pg/ml, respectively, thus indicating that we were unable to achieve effective total peripheral sympathectomy. This may also explain why Finch and Leach (1970) were unable to prevent development of one-kidney, one clip and one-kidney, DOCA, salt hypertension in rats by peripheral sympathectomy with intravenous 6-hydroxydopamine. In the study by Finch and Leach the rats were also not adrenal demedullated and plasma catecholamine levels were not reported in their experiments.

Our observation of normal norepinephrine levels in the sympathectomized rats may have been due to rapid regeneration of adrenergic nerve terminals in the 6-hydroxydopamine treated rats. Finch et al. (1973) report that vascular adrenergic nerve terminals can completely regenerate within seven days following intravenous 6-hydroxydopamine while adrenergic nerve terminals in other tissues completely regenerate within 14 to 28 days. It is likely then that the adrenergic nerve terminals had completely regenerated by the time of the terminal experiments in this study and therefore no conclusion regarding the role of the sympathetic nervous system on the development of reduced renal mass hypertension can be drawn.

None-the-less, some interesting conclusions can be made from our study. We can conclude that reduced renal mass hypertension can develop in the absence of adrenal medullary catecholamines. Also, since plasma catecholamine levels were not different in the control and experimental rats, this indicates that altered concentration of circulating catecholamines are not responsible for the elevated blood pressure in the experimental rats.

Central Sympathectomy

Our measurements of brain catecholamines in centrally sympathectomized reduced renal mass rats indicate that the sympathectomy was effective. Our findings suggest that central sympathectomy by intraventricular injections of 6-hydroxydopamine (prior to subtotal nephrectomy and substitution of saline for drinking) prevents the appearance of ouabain-like factor in plasma, prevents inhibition of vascular Na^+-K^+ pump activity, and prevents the development of hypertension. These data thus indicate that central adrenergic pathways are involved in the development of this type of hypertension and that central sympathectomy may prevent the development of this hypertension by interfering with the synthesis and/or release of the ouabain-like factor. These results further indicate that release of this factor is probably necessary for the development of reduced renal mass hypertension.

The observation that central sympathectomy prevented the development of hypertension in subtotally nephrectomized saline-drinking rats is in agreement with findings in other low renin, investigator-induced models of experimental hypertension. Haeusler et al. (1972) showed that central sympathectomy by intraventricular injections of 6-hydroxydopamine prevented the development of one-kidney, one clip and one-kidney, DOCA, salt hypertension in rats, thus further indicating a common pathophysiological mechanism of elevated blood pressure in low renin, investigator-induced models of experimental hypertension.

AV3V Lesions

Our studies with AV3V lesioned, subtotally nephrectomized rats show that electrolytic lesions of the AV3V region of the brain prevents both the appearance of ouabain-like factor in blood and the devel-

opment of reduced renal mass-saline hypertension. Na^+-K^+ pump activity was also measured in six pairs of AV3V lesioned (saline drinking) and sham lesioned (saline drinking) reduced renal mass rats. The results show that vascular Na^+-K^+ pump activity was lower in the sham lesioned rats in five of the six pairs. This difference however was not statistically significant, possibly because of the small sample size.

The finding that AV3V lesions prevent the development of reduced renal mass hypertension is consistent with findings in other low renin, investigator-induced models of hypertension. Fink et al. (1977) showed that AV3V lesions prevent the development of one-kidney, DOCA, salt hypertension and Buggy et al. (1977) showed that AV3V lesions prevent the development of one-kidney, Grollman wrap hypertension in rats. Also, Brody et al. (1978) showed that AV3V lesions reverse one-kidney, Grollman wrap hypertension. Brody et al. (1980) have also reported that AV3V lesions prevent the development of hypertension in high-salt-fed Dahl S rats but not in spontaneously hypertensive rats (SHR).

Our observation that AV3V lesions prevent the appearance of a ouabain-like factor in the plasma of saline-drinking reduced renal mass rats is supported by findings from other studies. Bealer et al. (1979) showed that AV3V lesions prevent the appearance of natriuretic factor in plasma following acute volume expansion of rats. Also, Pamnani et al. (1981a) reported that AV3V lesions abolish the inhibition of vascular Na^+-K^+ pump activity and the appearance of circulating ouabain-like factor in plasma of acuely volume expanded rats. Furthermore, Songu-Mize et al. (1981) showed that AV3V lesions prevent both the development of one-kidney, DOCA, salt hypertension and the inhibition of vascular Na^+-K^+ pump activity in tail arteries of these rats.

Collectively, these studies provide strong evidence that the AV3V region of the brain and central adrenergic pathways are involved in the synthesis or release of a sodium transport inhibiting factor(s) which plays a role in the development and maintenance of some types of experimental hypertension in rats. Studies involving electrical or chemical stimulation and ablation of specific areas in the AV3V region are needed to identify the centers controlling the synthesis and/or release of this factor.

ROLE OF VOLUME

Experiments of Buckalew and Nelson (1974) indicated that extracellular fluid vloume expansion might play a role in the release of a humoral ouabain-like agent. They showed that ultrafiltrates of jugular vein plasma from acutely volume expanded dogs inhibits short circuit current when applied to the toad bladder or frog skin and causes natriuresis when injected into assay rats. Pamnani et al. (1978b) showed that compared to sham-expanded rats, tail arteries from acutely volume expanded rats have significantly decreased Na^+-K^+ pump activity. Additionally, supernates of boiled plasma from volume expanded rats significantly decrease ^{86}Rb uptake when applied to normal rat tail arteries. These studies indicate that volume expansion might be a stimulus for the release of oubain-like factor into blood.

Although the volume status of several of the low renin, investigator-induced models of hypertension is an unresolved issue, the data presented in this study, in conjunction with data reported by other investigators, clearly show that the reduced renal mass-saline model of hypertension is volume expanded.

Also, although volume expansion appears to be a stimulus for the

release of the ouabain-like factor, an absolute increase in volume may not be necessary to trigger its release. Epstein (1978) has shown that immersion of normal subjects in water up to the neck causes the release of a natriuretic factor which can be recovered in the urine and blood. Since this maneuver causes distention of the pulmonary vascular bed this indicates that cardiopulmonary volume receptors may be involved in the release of circulating sodium transport inhibiting factor. This data also indicates that a shift of blood from the peripheral to the central (thoracic) circulation can elicit increases in the release of sodium transport inhbitor without accompanying changes in total absolute plasma or extracellular fluid volumes.

NATURE OF THE HUMORAL AGENT

The nature of the ouabain-like factor is not known. We do know that the humoral ouabain-like agent found in animals with low renin, investigator-induced models of experimental hypertension is heat stable as it is present in supernates prepared from boiled plasma.

Buckalew and Nelson (1974) have shown that the ouabain-like humoral factor found in the plasma of acutely volume expanded dogs has a molecular weight of less then 500. Gruber and Buckalew (1978) have isolated a natriuretic factor from the serum of acutely volume expanded dogs by passing their plasma through a Biogel P-2 column, collecting the peak that elutes after the salt peak, and passing this through high pressure liquid chromatography. This serum fraction inhibits short circuit current in the toad bladder and is natriuretic when injected into rats. Gruber and Buckalew suggest that this natriuretic hormone is a small acidic peptide (molecular weight less than 500) which is the active breakdown product of a larger precursor molecule. Furthermore,

Gruber et al. (1980) showed that plasma extracts from volume expanded dogs contains a factor which cross-reacts with an antibody to digoxin.

In contrast to these findings, Clarkson et al. (1980) have obtained a partially purified, non-peptide, small (less than 500 molecular weight), polar substance from the urine of normal subjects that causes natriuresis when injected into rats. The level of this substance increases when the sodium intake increases. Clarkson et al. speculate that this substance is a sugar attached to a ring.

Haupert and Sancho (1979) have obtained a low molecular weight, non-peptide substance from bovine hypothalamus which inhibits both short circuit current in toad bladder and renal Na^+, K^+-ATPase activity.

The chemical characterization of this humoral ouabain-like factor is yet to be determined.

SUMMARY AND CONCLUSIONS

Our studies show that increasing the sodium intake of subtotally nephrectomized rats by substitution of a one percent sodium chloride solution for drinking results in the development of hypertension and this is a low renin, volume expanded model of hypertension. Our data also indicate that cardiovascular muscle cell Na^+-K^+ pump activity is decreased in tail arteries and myocardium of reduced renal mass hypertensive rats due to the action of a circulating ouabain-like agent. The time course of the initiation of pump suppression and the development of hypertension further indicates that the decreased Na^+-K^+ pump activity may play a causal role in the genesis of the elevated blood pressure in these rats. Additionally, our studies with centrally sympathectomized and AV3V lesioned reduced renal mass rats indicate that central adrenergic pathways and the AV3V region of the brain are involved in the synthesis and/or release of the circulating ouabain-like agent and that integrity of these systems is necessary for the development of reduced renal mass hypertension.

The findings of this thesis project support the hypothesis that inhibition of cardiovascular muscle cell Na^+-K^+ pump activity by a humoral ouabain-like agent may be a common defect in several low renin, volume-dependent types of hypertension.

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